Hows and whys of left and right

Ontogeny of lateralization and its functional relevance

university of groningen

faculty of mathematics and natural sciences

NWO
Netherlands Organisation for Scientific Research

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SIXTH FRAMEWORK PROGRAMME

The research reported in this thesis was carried out at the Department of Behavioural Biology, University of Groningen, The Netherlands. All studies were approved by the Ethical Committee of the University of Groningen. The research was financially supported by the Sixth Framework Programme of the European Union to Prof. dr. Groothuis and the Faculty of Mathematics and Natural Sciences of the University of Groningen. Additional support was obtained from the Gratama stichting and the Netherlands Organisation for Scientific Research (NWO) to Prof. dr. Groothuis and the Reseach School of Behavioural and Cognitive Neurosciences (BCN).
The production of this thesis was financially supported by the Graduate School of Science (GSS) and the Faculty of Mathematics and Natural Sciences, University of Groningen.

Lay-out and figures:	Sara Schaafsma
Cover illustration :	Marcel Ruijters
Photos:	Sara Schaafsma unless otherwise stated
Printed by:	Ipskamp Drukkers

ISBN: 978-90-367-5236-7
ISBN: 978-90-367-5235-0 (electronic version)

RIJKSUNIVERSITEIT GRONINGEN

Hows and whys of left and right

Ontogeny of lateralization and its functional relevance

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken,
in het openbaar te verdedigen op
vrijdag 6 januari 2012
om 14:30 uur

door

Sara Marie Schaafsma

geboren op 24 december 1980
te Amsterdam

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General introduction

Sara M Schaafsma

Introduction

The human brain consists of two cerebral hemispheres. Cerebral lateralization refers to the asymmetric distribution of functions over both hemispheres. One of the most renowned expressions of cerebral lateralization is the lateralization of language; ninety-five percent of right-handed humans show left hemispheric dominance for language (Corballis 2003). Interest in functional brain lateralization started in 1861 with the observation by Pierre Paul Broca that two patients with severe language deficits showed injuries in the left posterior inferior frontal gyrus region in the frontal lobe of the human brain, an area later known as Broca's area. Although research at the beginning of the 20th century had already shown that structural asymmetries in the brain were present in a variety of vertebrates (for references see Vallortigara & Bisazza 2002), lateralization was later ascribed to humans solely and was thought to be hallmark of human evolution. Rediscovery of the extensiveness of the phenomenon of lateralization throughout the animal kingdom started with the finding in canaries that sectioning the left tracheosyringeal nerves had major differential effects on song production from sectioning the right tracheosyringeal nerves (Nottebohm 1971). Subsequently, lateralization was found to be present in chickens (e.g. Rogers & Anson 1979), in rodents (Denenberg *et al*. 1978, Glick & Ross 1981), and in cats (Webster 1977). Since then many studies have shown that lateralization is a fundamental aspect of the organization of brain and behaviour in vertebrates, even showing consistency of lateralization of functions over the hemispheres across species (e.g. Vallortigara & Bisazza 2002). This finding opened up new perspectives in the study of lateralization, as animal models could give us new insights into the ultimate and proximate mechanisms underlying lateralization. In order to investigate the evolution of neural and morphological lateralization using a comparative approach, combining ethological, developmental, and theoretical research, the research program 'Evolution and Development of Cognitive, Behavioural and Neural Lateralization' was set up and funded by the European Commission. Eight universities were part of this program and in this PhD thesis I present studies performed within this framework.

Lateralization of functions over the hemispheres is a heritable trait, but shows substantial developmental plasticity. In the first part of this thesis I address questions related to the development and causation (proximate mechanisms) of lateralization. Lateralization has been shown to improve brain efficiency, as it saves neural space by avoiding replication of functions and it allows simultaneous processing of different processes avoiding hemispheric competition (Rogers 2002). Hence, there is scientific consensus that lateralization is beneficial to the individual, although this benefit may be task dependent (e.g Boles *et al*. 2008). Although lateralization at the individual level is hypothesized to be beneficial, the fact that the majority of individuals within vertebrate species show the same direction of lateralization (population level lateralization) can not be explained by the abovementioned theories. In the second part of this thesis I address the ultimate mechanisms (function and evolution) underlying population level lateralization.

In the first part of this thesis my co-authors and I review the evidence for a genetic basis of lateralization, which is addressed mainly in the human literature. Next, we explore the evidence of lateralization being a plastic trait and the factors underlying this plasticity. After this large review, we report two studies on the ontogenetic plasticity of lateralization. Lateralization comprises two aspects: direction and strength. Direction defines whether an individual shows left- or right-sided lateralization, whereas strength of lateralization defines the degree to which an individual deviates from no lateralization, independent of the direction of lateralization. Sex differences in both direction and strength of lateralization have been observed repeatedly in humans; males are more often left-handed (Papadatou-Pastou *et al.* 2008) but right-handed men are stronger lateralized than right-handed women (Bourne 2005 and references therein). As males are exposed prenatally to higher levels of testosterone than females, testosterone is an often mentioned candidate to affect lateralization. Two experimental studies are conducted to test the effects of prenatal and postnatal testosterone on lateralization in a cichlid fish species. In these studies cichlid fish are used for various reasons. First, cichlid fish are oviparous and prenatal testosterone can be easily manipulated by bathing the eggs in a hormone solution. Second, cichlids have laterally placed eyes and small overlapping visual fields facilitating easy determination of lateralization in viewing tasks. In addition to hormonal factors, other factors may also influence lateralization. One such factor is training. Increased training of one side of the body over the other may affect lateralization two-fold; training may affect both the preference to use one side of the body and the asymmetry of skill of each side of the body in performing a certain task. The associations between training and these different aspects of lateralization are difficult to investigate in animal species, but school attendance in humans is an excellent model system as specific tasks, such as writing, are substantially and daily trained. We therefore investigate the effect of training on lateralization in a human population in which a vast proportion of the population did not attend school: a non-industrial population in the Eipo valley, Papua, Indonesia. In 1981 an elementary school was built in the Eipo valley. No fees are required to attend this school, but many children do not attend school for various reasons (e.g. to be able to help their parents in the gardens). This gives us the unique opportunity to investigate the association between schooling and handedness.

In the second part of this thesis we address the function and evolution of lateralization. In humans, cerebral lateralization of fine motor skill results in contralateral handedness. Benefits of lateralization at the individual level have been shown to be present as strength of lateralization is associated with increased performance in several tasks (Boles *et al.* 2008). However, it remains unclear whether or not it is beneficial to show lateralization in the same direction as the majority of the population. In vertebrates, this so-called population level lateralization is present in many different behaviours. Human handedness is the most exceptionally skewed lateralized behaviour known in the animal kingdom, with only about 4-13% left-handers (Perelle & Ehrman 1994; Raymond *et al.* 1996). Investigating this specific lateralized behaviour may have a high potential in revealing the function and evolution of population level lateralization.

Left-handedness, a heritable trait, is associated with certain costs related to health, possibly leading to a decrease in Darwinian fitness. One evolutionary hypothesis, the fighting hypothesis, has been proposed to explain the persistence of left-handedness

(Raymond *et al.* 1996). This hypothesis states that left-handedness can be maintained in populations because left-handers have a frequency-dependent advantage in fights. However, whether these health related costs (see below) will actually lead to decreased Darwinian fitness, and thereby making handedness subject to natural selection pressures, is unknown. Furthermore, the associations between costs and left-handedness are found in Western societies, which may no longer be under the same selection pressures in which handedness has evolved. In order to investigate the fighting hypothesis and advantages and disadvantages of phenotypes of lateralization in relation to Darwinian fitness in a current society which most resembles human societies in which handedness originally evolved, we aim to measure handedness and key components of Darwinian fitness in individuals living in a non-industrial society: the Eipo. The Eipo inhabit an area of around 150 square kilometres along the banks of the Eipomek river at approximately 4°25`-4°27` S, 140°00`-140°05` E. in the highlands of the Indonesian province of Papua, formerly known as Irian Jaya (New Guinea). The inhabitants are horticulturists whose staple food consists of sweet potatoes and vegetables, complemented by the products of hunting, gathering and pig raising (Schiefenhövel 1976, 1991; personal observations 2009). Because of the remoteness and inaccessibility of the Eipo valley (at present the area is accessible by foot or light aircraft only), it has until recently been isolated from the outside world.

Proximate factors underlying lateralization

Despite several decades of research, the ontogeny of lateralization of brain and behaviour is still elusive, although it is important in understanding its developmental plasticity, function and evolution, and its relationship with developmental disorders (such as autism spectrum disorder, Kleinhans *et al.* 2008). Although it is apparent that lateralization is a heritable trait, with heritability estimates varying between 0.23 and 0.66 (Denny & Sullivan 2007), the differential contributions of genetic and non-genetic factors underlying this heritability are still far from clear. In **chapter 2** my co-authors and I review the evidence of the genetic basis underlying lateralization. First, we discuss the explanatory power of the predominant genetic models of human handedness, the most obvious expression of cerebral lateralization in behaviour. After addressing the strengths and weaknesses of these models we discuss the evidence for plasticity of lateralization driven by modulating factors such as social pressures, parental effects and asymmetric input of stimuli in human and nonhuman vertebrate species.

Prenatal testosterone

After reviewing the ontogeny of lateralization we further explore the plasticity of lateralization by experimentally investigating the effects of the gonadal steroid hormone testosterone on behavioural lateralization. Testosterone has often been suggested to affect lateralization, a view driven by the small but persistently found difference in lateralization patterns between the sexes, with men being more often left-handed (for a meta-analysis see Papadatou-Pastou *et al.* 2008). This finding has lead researchers to postulate

hypotheses regarding the effect of prenatal testosterone on lateralization. One well cited hypothesis, put forward by Geschwind & Galaburda (1985), proposes that prenatal testosterone slows down neuronal growth in the left hemisphere and consequently leads to compensatory growth in the right hemisphere. Increased levels of prenatal testosterone would thus lead to increased dominance of the right hemisphere and therefore induce more left-handedness. This theory has been extensively criticized in the literature (e.g. Bryden *et al.* 1994). An alternative hypothesis, the callosal hypothesis, states that prenatal testosterone increases axonal pruning in the corpus callosum, at least in males, which leads to a decrease of communication between the hemispheres and therefore increases the strength of lateralization (Witelson & Nowakowski 1991). Finally, the sexual differentiation hypothesis proposes that lateralization is related to characteristics of the process of sexual differentiation and that early exposure to testosterone causes masculinisation of lateralization (Witelson & Nowakowski 1991). Evidence supporting any of these theories is scarce and sometimes contradictory (for meta-analyses see Pfannkuche *et al.* 2009), possibly caused by the use of non-random clinical samples (e.g. individuals with Congenital Adrenal Hyperplasia (CAH), or daughters of women treated with Diethylstilbestrol (DES) during pregnancy) or indirect markers for prenatal testosterone (e.g. 2D:4D digit ratio, postnatal testosterone levels, or the sex of the co-twin). In **chapter 3**, we investigate the effect of prenatal testosterone on behavioural lateralization, and the differential effect it has on the two sexes, by conducting an experiment on a random healthy sample using the cichlid fish *Aequidens rivulatus*. Prenatal testosterone may originate both from the foetus itself and from its mother. Fish eggs contain substantial levels of maternal androgens (Schreck *et al.* 1991) which are known to influence offspring development (Gagliano & McCormick 2009). Since maternal hormone production is under the influence of the environment, a mother can communicate the prevailing conditions to her offspring by means of depositing different amounts of gonadal hormones to her eggs. In this experiment we collected eggs immediately after spawning and treated half of the eggs with methyl-testosterone, an androgen that has a high affinity to testosterone receptors and is stable in water, and the other half with the solvent only. We measured whether the testosterone treatment, mimicking elevated deposition of maternal androgens, affected lateralization in these fish at the age of 7 months, when the fish are sexually mature, in two behavioural tasks. In the first task we investigate the preference of individuals to inspect a predator, which fish generally do with their right eye (Bisazza & Vallortigara 1997; Vallortigara *et al.* 1999; Facchin *et al.* 1999; Brown *et al.* 2004). The second task is more socially oriented, as we investigate the preference of individuals to view their mirror image which is perceived as a conspecific (e.g. Bisazza *et al.* 1999) which fish generally do with their left eye (Sovrano *et al.* 1999, 2001; de Santi *et al.* 2001; Sovrano & Andrew 2006). In this way we investigate whether the hormone testosterone, in the prenatal stage of life, influences the direction (left versus right) and the strength (independent of direction) of lateralization of viewing preference. We sacrificed the fish after the tests to histologically determine the sex, enabling us to analyse sex-specific effects of our treatment.

Postnatal testosterone

Although most research concerning the influence of testosterone on lateralization has focused on its effect during the prenatal stage, testosterone may also affect lateralization in a later stage of life. Human testosterone levels fluctuate over the day and over the year and have been used to investigate the short term (activating) effects of testosterone on spatial ability. Spatial ability is positively associated with cerebral lateralization and therefore used as an indirect measure of lateralization (Gouchie & Kimura 1991; Kimura & Toussaint 1991; Moffat & Hampson 1996a). Experimental evidence, obtained from older men and female-to-male transsexuals receiving testosterone supplements, indicates that testosterone increases spatial cognition (reviewed in Cherrier 2009), presumably due to an increase in strength of lateralization. Others have tried to correlate circulating levels of testosterone with handedness (Tan 1991; Moffat & Hampson 1996b; Moffat & Hampson 2000; Gadea 2003; Beaton *et al.* 2010), a more direct measure of lateralization, but results are ambiguous. Indirect evidence in cichlid fish indicates that postnatal testosterone can affect lateralization. Adult fish show a sex difference in lateralization which is associated with their level of aggressiveness (Reddon & Hurd 2008). As aggressiveness and testosterone levels correlate in fish (Munro & Pitcher 1985; Higby *et al.* 1991), these results suggest that testosterone may activate sex differences in lateralization.

In **chapter 4** we investigate whether postnatal testosterone affects behavioural lateralization sex-specifically. We experimentally increased levels of testosterone in six month old fish by treating them for two months with methyl-testosterone (or solvent only for the control group) which was administrated to the water in their home tanks. After this treatment we established behavioural lateralization of eye use during predator viewing using the same apparatus as in chapter 3.

Schooling

In addition to gonadal hormones, other factors may also influence lateralization. A slight preference to use a certain side of the body early in life (as has been shown to be the case in human foetuses) may be reinforced during development due to increased practice of the preferred hand over the other. Besides this internal factor, external factors such as social pressure may also play a role in the ontogeny of lateralisation. Training of one side of the body could affect lateralisation two-fold. It could change the asymmetry in skill of the sides of the body and it could change the preference to use a certain side of the body. These possibly interconnected associations are difficult to test in animals, but in humans it can be investigated when studying the association between institutionalized cultural processes such as formal education and handedness. Formal education involves repetition of specific motor tasks (such as writing) with one hand. This training in school may increase strength of lateralization. In addition, it has been postulated that acquisition of reading and writing specifically may change the brain's organization which is supported by results which indicate that the corpus callosum, a bundle of neuronal fibres connecting the two hemispheres, is thicker in literate than in illiterate individuals (Castro-Caldas *et al.* 1999). Furthermore, illiterate subjects are consistently more right-lateralized in the inferior parietal cortex (which correlates with degree of left-handedness) than literate controls (Petersson *et al.* 2007). As the effect of schooling on lateralization is difficult to investigate in a Western population as most children attend school, Connolly & Bishop

(1992) investigated the association between schooling and a specific form of lateralization, handedness, in a Papuan society, in which only a part of the population ever entered school. They found no differences concerning handedness between schooled and non-schooled subjects, contradicting the prediction that learning to write would influence an individual to become more right-handed on pencil use and other related activities. However, they investigated hand preference with a battery of tests suited for a Western society, tests unfamiliar to the subjects, thereby possibly making it an erroneous measurement to indicate lateralization. To investigate the effect of schooling on handedness one should investigate hand preference based on ecologically relevant tasks. In **chapter 5** we explore the relationship between schooling and hand preference in a population in which a vast proportion of the society did never attend school, a non-industrial population in Papua (Indonesia), the Eipo. Hand preference is based on 10 ecologically relevant tasks, such as throwing and bush knife usage, that the subjects had to perform. Additionally, in this chapter we explore the relationship between schooling and three aspects of asymmetry of hand skill. First, we measured the asymmetry in speed of fine motor control of both hands by means of Annett's pegboard task (Annett 1985, p. 208). Second, we measured the accuracy of throwing and finally we measured asymmetry of hand grip force. Information on schooling of the subjects was obtained through questionnaires, while the subject's age, unknown to the subject, was estimated by the investigator based on a series of major events in the community of which exact dates were known from documented records that the subjects recollected. Learning to write entails practicing one hand in fine motor skill. Therefore, we expect that schooling leads to an increase in asymmetry of fine hand performance (measured with the pegboard task) and an increase in hand preference and possibly to increased right-handedness both on hand preference and asymmetry of hand skill due to overt or covert pressures by the environment (e.g. teachers).

Ultimate factors underlying lateralization

In the second part of this thesis I address a different matter, namely the functional relevance of a particular type of lateralization, human handedness, and the persistence of different phenotypes of handedness. As mentioned above, handedness is a heritable trait and left-handedness is associated to potential health problems (see below). These potential health problems may be associated with a decrease in Darwinian fitness. If associations between handedness and Darwinian fitness exist, natural selection can thus act on the trait of handedness.

Historical perspectives on left-handedness

Historically, left-handedness has often been portrayed as an undesirable characteristic. Cesare Lombroso (1835-1909), a Turin physician believed to be a cutting edge scientist at the time, developed a theory concerning the causes of criminality, insanity, feeblemindedness, and left-handedness called 'avatism'. He claimed that these characteristics arise due to the regression to primitive mentalities.

As

'man advances in civilization and culture he shows an always greater right-sidedness as compared to...women and savage races...'

(quoted by Kushner 2011). Of course these views are outdated and prejudiced, but the word 'left' still has negative connotations in many expressions and languages in many cultures. For example, the Latin word sinistra originally meant left but took the meaning of evil and unlucky. This negative connotation is still present in the English word 'sinister', whereas the word 'right' is associated with proper and correct, similar to the meaning of the Slavic root word for 'right': 'prav' used in words with meanings like correctness and justice. Furthermore, the Bible is riddled with numerous unfavourable references towards left-handedness. Direct social pressures to use the right hand for acts such as eating and writing are still present in many societies. Additionally, there may be indirect and subtle pressures operating upon left-handers as the human made world is designed for right-handers, and machines and tools (such as scissors) are adjusted to fit the majority of people (Porac & Coren 1981). Although prejudices and overt or covert pressures make it harder to conduct proper scientific research on the proximate and ultimate mechanisms underlying handedness, studies have tried to investigate the phenomenon of handedness objectively and these studies also suggest that left-handedness, or non-right-handedness, is not a neutral trait.

Costs and benefits

Left-handedness is associated with a reduction in components of Darwinian fitness, such as decreased lifespan (although this claim is controversial), stress during birth and accompanied problems such as extremely low birth weights (for references see Searleman *et al.* 1989; Llaurens *et al.* 2009). Neuronal deficiency due to birth stress leading to increased chances of becoming left-handed may be caused by oxygen deficiency. The left hemisphere has also been hypothesized to be more susceptible to damage than the right hemisphere (reviewed in Liederman 1983), also possibly due to the idea that the left hemisphere matures more slowly and therefore is longer at risk when in an adverse intrauterine environment (Geschwind & Galaburda 1985; chapter 3). Coren & Halpern (1991) built on this idea and hypothesized that left-handedness may be a marker for mild instances of neuropathology. Left-handedness has been found to be associated with low cognitive development (e.g. Miller 1971; Siengthai *et al.* 2008, but see e.g. van der Elst *et al.* 2008), delayed maturation (Coren *et al.* 1986), developmental disorders (Kleinhans *et al.* 2008), and autoimmune diseases (Searleman & Fugagli 1987; Morfit & Weekes 2001).

However, if left-handedness were heritable and associated with reduced fitness one would expect it to go extinct. Obviously, this has not happened, possibly because it has advantages as well. Indeed studies have shown that left- or non-right-handedness is also associated with benefits that may positively affect fitness. It is related to increased socioeconomic status (Faurie *et al.* 2008) and an increased prevalence of left-handers among (instrumental) musicians (Byrne 1974; Peterson 1979; Götestam 1990; Hassler & Gupta 1993; Aggleton *et al.* 1994), artists (Peterson 1979; Preti & Vellante 2007;), and in the top of combative sports such as fencing (e.g. Wood & Aggleton 1989; Raymond *et al.* 1996; Harris 2010) has been observed. This latter finding has been long known, and came about in poetry about the Kerrs, the sixteenth-century Scottish border reivers (border raiders):

The deadliest foes
That e'er to Englishmen were known
For they were all bred left-handed men
And fence against them there was none

Also, Italian fencing master Camillo Palladini of Bologna had allegedly addressed the advantage of left-handedness in fencing in his treatise Discorso Sopra l'Arte Della Scherma (Discourse on the art of fencing):

touch[ing] on the subject of left-handed adversaries many believe that a left-hander has an advantage over a right-hander

(c. 1560; taken from Harris 2010). The finding that left-handers have an advantage in interactive sports is the most interesting of all benefits associated to left-handedness, as it can theoretically explain the polymorphism of handedness with left-handers always being in the minority (Raymond *et al.* 1996). Left-handers could have an advantage in interactive sports, not because they would be intrinsically better at sports (although some evidence has been found for this idea as well, reviewed in Harris 2010), but because they are in the minority. Raymond *et al.* (1996) postulated the fighting hypothesis: left-handers have a frequency-dependent advantage in fights and this could, in harmony with the increased costs concerning health, lead to the persistence of left-handedness due to an evolutionary stable strategy when fighting is important in societies. They investigated this idea by performing a cross-cultural comparative study among eight non-industrial societies (Faurie & Raymond 2005). They found a positive correlation between number of homicides committed and percentages of left-handedness. However, the data concerning handedness collected were mostly based on indirect evidence and need to be investigated in further detail.

The fighting hypothesis

To further investigate the persistence of left-handedness and whether this can be explained by the fighting hypothesis raised by Raymond and colleagues (1996), we measured handedness in the non-industrial Eipo population. In the Eipo valley, tribal warfare and intra-communal fights were still common when missionaries began their work in the valley in 1978 (Ploeg, 2004). The fighting hypothesis predicts that the frequency of left-handedness is very high in this population, as fighting is important (Faurie & Raymond 2005) and is predicted to be higher than in a Western population in which fighting may occur less frequently and violence is more often conducted with long range weapons in stead of hand to hand combat. Indirect evidence indicated that the frequency of left-handers in the Eipo population is indeed very high (20.4%, Faurie & Raymond 2005). In **chapter 6**, we measure the frequency of left-handers in the Eipo population in a detailed manner in order to obtain a more reliable measure of handedness in this population. Furthermore, we compare the frequency of left- and mixed-handed Papuans with a Western sample. The Western sample comprises Dutch biology students who executed the same tasks as did the Papuans. To investigate whether the student sample is representative for the Western population we compare our results to data collected by Annett (2004) who measured handedness in a Western population with an inventory based on tasks common to Western daily life. As tribal wars came to an end and homicide rate

diminished dramatically due to the acceptance of Christianity as the primary religion around 1980 (Ploeg 2004), we test whether handedness differs between people who were adults in the period when tribal wars were common and people who were too young to take part or not yet born. This analysis is repeated for men only, as only they participate in the fights and women would benefit of being left-handed only indirectly, via their left-handed sons.

Handedness and reproductive success

In investigating the costs and benefits of different lateralization phenotypes, widely varying methods have been used to categorize individuals according to handedness. Some researchers categorized left- and right-handers, whereas others categorized non-right- versus right-handers or left-, right- and ambidextrous (approximately equally adept with both hands) groups, thereby causing confusion in the field. Furthermore, categorizing individuals in two or three groups does not take into account the fact that direction and strength of lateralization are intertwined. Nettle (2003) showed that findings of differences between left- and right-handers could be attributed to the fact that left-handers are generally less strongly lateralized than right-handers. Therefore, strength and direction of lateralization should be both considered in order to differentiate between the effects of these two aspects of lateralization. In addition, handedness is most often investigated in terms of preference (which hand is preferably used for a certain task), although asymmetry of skill between the hands may be equally important, as this may be the more likely trait to be under natural selection pressures. As hand preference and asymmetry of hand performance show only a weak correlation (Triggs *et al*. 2000; Doyen *et al*. 2008) they may represent different aspects of lateralization.

Phenotypes of handedness are related to costs and benefits, but whether they actually can affect Darwinian fitness is not known. In a Western population, one study found a trend for left-handers to have fewer offspring than right-handers (Faurie *et al*. 2006), but this was a non-significant trend and as only direction of handedness was investigated, this study could not differentiate between direction and strength of handedness. Furthermore, all studies investigating the costs and benefits of handedness were performed in Western societies (except for the cross-cultural literature study by Faurie & Raymond, 2005). These societies are likely to not be under the same selection pressures in which handedness has evolved. In order to investigate the association between handedness and Darwinian fitness in a current society which most resembles human societies in which handedness originally evolved, we aim to measure handedness and reproductive success and health (key components of Darwinian fitness) in individuals living in a non-industrial society, the Eipo population. This population is one of the populations in which a high percentage of left-handers was recorded by means of indirect measurement in the Faurie and Raymond (2005) study. In **chapter 7**, we investigate the relationship between handedness and reproductive success in this Papuan population. We analysed the differential explanatory power of direction and strength of hand preference and asymmetry in hand skill (in speed of fine motor control and accuracy of throwing) on measures of reproductive success. Furthermore, we investigate whether the associations found were mediated by the health of the subjects. Data on reproductive success and health were obtained through interviews. We examine the relationship between the direction

and strength of handedness with the number of children born to a mother or father, the number of children that were still alive during the field work period and the number of children that died before they reached the age of two years. By examining the associations between various aspects of handedness and several facets of reproductive success, we can investigate the differential contributions of patterns of handedness to various components of reproductive success, even though the net fitness may be equal between groups due to a balancing selection mechanism such as negative frequency-dependent selection as was postulated by Raymond *et al*. and colleagues (1996).

The health care hypothesis

As we found low percentages of left-handers in the Papuan population investigated in this thesis (chapter 6), a population in which health care was absent until 2005, and as many studies have found associations between left-handedness and reduced health (see above) we speculate that possibly health care, in addition to or alternative to the fighting hypothesis, could influence the percentages of left-handedness in Western societies. In **chapter 8**, we investigate this idea with a cross-national approach in which we correlate homicide rates and public expenditures on health care with levels of left-handedness. In this study we use data collected by Perelle & Ehrman (1994) on handedness of 12 Western countries. We correlate these data with numbers of homicides, of which data were taken from the United Nations Survey of Crime Trends and Operations of Criminal Justice Systems. We also correlate the percentage of left-handers in Western societies with public expenditure on health care, of which data were obtained through the Organisation for Economic Co-operation and Development. Taking this approach we can test which variable has greater explanatory power and thus investigate whether either the fighting hypothesis or the health care hypothesis can better explain the variation in left-handedness in Western societies.

Finally, in **chapter 9**, I will integrate the results presented in the chapters of this thesis.

References

Aggleton, J.P., Kentridge, R.W. & Good, J.M.M. 1994. Handedness and Musical Ability: A Study of Professional Orchestral Players, Composers, and Choir Members. *Psychology of Music*, **22(2)**, 148-156

Annett, M. 1985. Left, Right, Hand and Brain: The Right Shift Theory. Erlbaum : London

Annett, M. 2004. Hand preference observed in large healthy samples: Classification, norms and interpretations of increased non-right-handedness by the right shift theory. *British Journal of Psychology*, **95**, 339-353.

Beaton, A.A., Rudling, N., Kissling, C., Taurines, R., Thome, J. 2010. Digit ratio (2D:4D), salivary testosterone, and handedness. *Laterality*, **16(2)**, 135-155.

Bisazza, A. & Vallortigara, G. 1997. Rotational swimming preferences in mosquitofish: Evidence for brain lateralization? *Physiology & Behavior*, **62(6)**, 1405-1407.

Bisazza, A., de Santi, A., & Vallortigara, G. 1999. Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal behaviour*, **57(5)**, 1145-1149.

Boles, D.B., Barth, J.M. & Merrill, E.C. 2008. Asymmetry and performance: toward a neurodevelopmental theory. *Brain and cognition*, **66(2)**, 124-39.

Bourne, V.J. 2005. Lateralised processing of positive facial emotion: sex differences in strength of hemispheric dominance. *Neuropsychologia*, **43(6)**, 953-6.

Brown, C., Gardner, C. & Braithwaite, V. 2004. Population variation in lateralized eye use in the poeciliid Brachyraphis episcopi. *Proceedings of the Royal Society B: Biological Sciences*, **271** Suppl, 455-457.

Bryden, M., McManus, I. & Bulman-Fleming, M.B. 1994. Evaluating the empirical support for the Geschwind-Behan-Galaburda model of cerebral lateralization. *Brain and Cognition*, **26(2)**, 103–167.

Byrne, B., 1974. Handedness and musical ability. *British Journal of Psychology*, **2**, 279-281.

Castro-Caldas, A., Miranda, P.C., Carmo, I., Reis, A., Leote, F., Ribeiro, C., Ducla-Soares, E. 1999. Influence of learning to read and write on the morphology of the corpus callosum. *European Journal of Neurology*, **6(1)**, 23–28.

Cherrier, M.M. 2009. Testosterone effects on cognition in health and disease. *Frontiers of hormone research*, **37**, 150.

Connolly, K.J. & Bishop, D.V. 1992. The measurement of handedness: a cross-cultural comparison of samples from England and Papua New Guinea. *Neuropsychologia*, **30(1)**, 13-26.

Corballis, M.C. 2003. From mouth to hand: Gesture, speech, and the evolution of right-handedness. *Behavioral and Brain Sciences*, **26(2)**, 199–208.

Coren, S. & Halpern, D.F. 1991. Left-handedness: A marker for decreased survival fitness. Psychological Bulletin, **109(1)**, 90–106.

Coren, S., Searleman, A. & Porac, C. 1986. Rate of physical maturation and handedness. *Developmental Neuropsychology*, **2(1)**, 17-23.

Denenberg, V.H., Garbanati, J., Sherman, D.A., Yutzey, D.A., Kaplan, R. 1978. Infantile stimulation induces brain lateralization in rats. *Science*, **201(4361)**, 1150.

Denny, K. & Sullivan, V.O. 2007. The economic consequences of being left-handed: Some sinister results. *Journal of human resources*, **42(2),** 353.

Doyen, A.L., Dufour, T., Caroff, X., Cherfouh, A., Carlier, M. 2008. Hand preference and hand performance: cross-sectional developmental trends and family resemblance in degree of laterality. *Laterality*, **13(2)**, 179-97.

Elst, W. van der, Boxtel, M.P. van, Breukelen, G.J. van, Jolles, J. 2008. Is left-handedness associated with a more pronounced age-related cognitive decline? *Laterality*, **13(3)**, 234-54.

Facchin, L., Bisazza, A. & Vallortigara, G. 1999. What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural brain research*, **103(2)**, 229–234.

Faurie, C. & Raymond, M. 2005. Handedness, homicide and negative frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, **272(1558)**, 25.

Faurie, C., Alvergne, A., Bonenfant, S., Goldberg, M., Hercberg, S., Zins, M., Raymond , M. 2006. Handedness and reproductive success in two large cohorts of French adults. *Evolution and Human Behavior*, **27(6)**, 457–472.

Faurie, C., Bonenfant, S., Goldberg, M., Hercberg, S., Zins, M., Raymond M. 2008. Socioeconomic status and handedness in two large cohorts of French adults. *British Journal of Psychology*, **99(4)**, 533–554.

Gadea, M. 2003. Salivary testosterone is related to both handedness and degree of linguistic lateralization in normal women. *Psychoneuroendocrinology*, **28(3)**, 274-287.

Gagliano, M. & McCormick, M.I. 2009. Hormonally mediated maternal effects shape offspring survival potential in stressful environments. *Oecologia*, **160(4)**, 657-665.

Geschwind, N. & Galaburda, A.M. 1985. Cerebral lateralization - Biological mechanisms, associations, and pathology: I-III A hypothesis and a program for research. *Archives of Neurology*, **42**, 428–459, 521–552, 634–654.

Glick, S.D. & Ross, D. 1981. Right-sided population bias and lateralization of activity in normal rats. *Brain research*, **205(1)**, 222-225.

Gouchie, C. & Kimura, D. 1991. The relationship between testosterone levels and cognitive-ability patterns. *Psychoneuroendocrinology*, **16(4)**, 323-334.

Götestam, K.O. 1990. Left-handedness among students of architecture and music. *Perceptual and motor skills* **70(3 Pt 2)**, 1323-1327.

Harris, L.J. 2010. In fencing, what gives left-handers the edge? Views from the present and the distant past. *Laterality*, **15(1)**, 15-55.

Hassler, M. & Gupta, D. 1993. Functional brain organization, handedness, and immune vulnerability in musicians and non-musicians. *Neuropsychologia*, **31(7)**, 655–660.

Hepper, P., Shahidullah, S. & White, R. 1991. Handedness in the human fetus. *Neuropsychologia*, **29(11)**, 1107-11.

Hepper, P., Wells, D. & Lynch, C. 2005. Prenatal thumb sucking is related to postnatal handedness. *Neuropsychologia*, **43(3)**, 313-315.

Higby, M., Beulig, A. & Dwyer, J. 1991. Exogenous testosterone and social experience each enhance the development of aggressive behavior in *Cyprinodon variegatus*. *Aggressive Behavior*, **17(4)**, 229-239.

Kimura, D & Toussaint, C. 1991. Sex differences in cognitive function vary with the season. *Society for Neuroscience Abstracts*.

Kleinhans, N.M., Muller R.A., Cohen D.N., Courchesne E. 2008. Atypical functional lateralization of language in autism spectrum disorders. *Brain Research*, **1221**, 115-125.

Kushner, H.I. 2011. Cesare Lombroso and the pathology of left-handedness. *The Lancet*, **377(9760)**, 118-119.

Liederman, J. 1983. Mechanisms underlying instability in the development of hand preference. In G. Young, S. Segalowitz, C.M. Carter, S.E. Trehub, eds. *Manual specialization and the developing brain.* New York: Academic Press. pp.321-330

Llaurens, V., Raymond, M. & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 881-894.

Miller, E. 1971. Handedness and the pattern of human ability. *British Journal of Psychology*, **62(1)**, 111-112.

Moffat, S.D. & Hampson, E. 1996a. A curvilinear relationship between testosterone and spatial cognition in humans: Possible influence of hand preference. *Psychoneuroendocrinology*, **21(3)**, 323-337.

Moffat, S.D. & Hampson, E. 1996b. Salivary testosterone levels in left- and right-handed adults. *Neuropsychologia*, **34(3)**, 225-233.

Moffat, S.D. & Hampson, E. 2000. Salivary testosterone concentrations in left-handers: An association with cerebral language lateralization? *Neuropsychology*, **14(1)**, 71-81.

Morfit, N. & Weekes, N. 2001. Handedness and immune function. *Brain and Cognition*, **46 (1-2)**, 209-213.

Munro, A.D. & Pitcher, T.J. 1985. Steroid hormones and agonistic behavior in a cichlid teleost, Aequidens pulcher. *Hormones and behavior*, **19(4)**, 353-371.

Nettle, D. 2003. Hand laterality and cognitive ability: A multiple regression approach. *Brain and Cognition*, **52(3)**, 390-398.

Nottebohm, F. 1971. Neural lateralization of vocal control in a passerine bird. I. Song. *Journal of Experimental Zoology*, **1777**, 229-262.

Papadatou-Pastou, M., Martin, M., Munafò,. M.R., Jones, G.V. 2008. Sex differences in left-handedness: a meta-analysis of 144 studies. *Psychological Bulletin*, **134(5)**, 677-699.

Perelle, I.B. & Ehrman, L. 1994. An international study of human handedness: the data. *Behavior Genetics*, **24(3)**, 217-227.

Peterson, J. 1979. Left-handedness: differences between student artists and scientists. *Perceptual and Mmotor Skills*, **48(3 Pt 1)**, 961-962.

Petersson, K.M., Liva, C., Castro-Caldas, A., Ingvar, M., Reis, A. 2007. Literacy: a cultural influence on functional left-right differences in the inferior parietal cortex. *The European Journal of Neuroscience*, **26(3)**, 791-799.

Pfannkuche, K.A., Bouma, A. & Groothuis, T.G.G. 2009. Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 929-942.

Ploeg, A. 2004. The German Eipo Research Project. *Journal de la Société des Océanistes*, **118**, 35-79.

Porac, C. & Coren, S. 1981. Lateral preferences and human behavior. New York: Springer.

Preti, A. & Vellante, M. 2007. Creativity and psychopathology: higher rates of psychosis proneness and nonright-handedness among creative artists compared to same age and gender peers. *The Journal of Nervous and Mental Disease*, **195(10)**, 837-845.

Raymond, M. Pontier, D., Dufour, A.M., Møller, A.P. 1996. Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society: Biological Sciences*, **263(1377)**, 1627–1633.

Reddon, A.R. & Hurd, P.L. 2008. Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters*, **4(4)**, 338-340.

Rogers, L.J. 2002. Advantages and disadvantages of lateralization. In L. Rogers & R. Andrew, eds. *Comparative vertebrate lateralization.* Cambridge: Cambridge University press, pp. 126-153.

Rogers, L.J. & Anson, J.M. 1979. Lateralisation of function in the chicken fore-brain. *Pharmacology, Biochemistry, and Behavior*, **10(5)**, 679-686.

Santi, A. de, Sovrano, V.A., Bisazza, A. & Vallortigara G. 2001. Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour,* **61(2)**, 305-310.

Schreck, C., Fitzpatrick, M.S., Feist G.W. & Yeoh, C.G. 1991. Steroids: developmental continuum between mother and offspring. In A. Scott, J.P. Sumpter, D.E. Kinne, M.S. Rolfe, eds. *Reproductive Physiology of Fish.* Proceedings Fourth International Symposium on the Reproductive Physiology of Fish. Sheffield: FishSymp., pp. 256-258.

Searleman, A. & Fugagli, A. 1987. Suspected autoimmune disorders and left-handedness: evidence from individuals with diabetes. Crohn's disease and ulcerative colitis. *Neuropsychologia*, **25(2)**, 367-374. 25(2), 367-374.

Searleman, A., Porac, C. & Coren, S. 1989. Relationship between birth order, birth stress, and lateral preferences: a critical review. *Psychological Bulletin*, **105(3)**, 397-408.

Siengthai, B., Kritz-Silverstein, D. & Barrett-Connor, E. 2008. Handedness and cognitive function in older men and women: a comparison of methods. *The Journal of Nutrition, Health & Aging*, **12(9)**, 641-647.

Sovrano, V.A & Andrew, R. 2006. Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research*, **167(2)**, 226–231.

Sovrano, V.A, Bisazza, A. & Vallortigara, G. 2001. Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiology & behavior*, **74(1-2)**, 237-244.

Sovrano, V.A. Rainoldi, C., Bisazza, A., Vallorigara, G. 1999. Roots of brain specializations: preferential left-eye use during mirror image inspection in six species of teleost fish. *Behavioral Brain Research*, **106**, 175-180.

Tan, U. 1991. Serum testosterone levels in male and female subjects with standard and anomalous dominance. *International Journal of Neuroscience*, **58(3-4)**, 211–214.

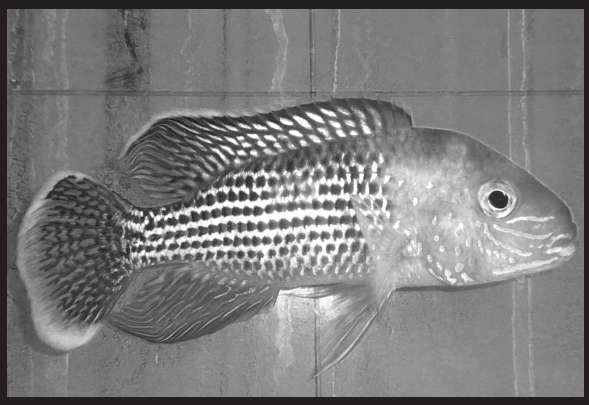
Triggs, W.J., Calvanio, R., Levine, M., Heaton, R.K. & Heilman1 K.M. 2000. Predicting hand preference with performance on motor tasks. *Cortex*, **36(5)**, 679-689.

Vallortigara, G. & Bisazza, A. 2002. How ancient is brain lateralization. In Lesley J Rogers & R. J. Andrew, eds. *Compartive vertebrate lateralization.* Cambridge, New York: Cambridge University Press, pp. 9-69.

Webster, W. 1977. Hemispheric asymmetry in cats. In S. Harnad *et al*. eds. *Lateralization in the Nervous System*. New York: Academic Press, pp. 471-480.

Witelson, S.F. & Nowakowski, R.S. 1991. Left out axons make men right: a hypothesis for the origin of handedenss and functional asymmetry. *Neuropsychologia*, **4**, 327-333.

Wood, C. & Aggleton, J. 1989. Handedness in "fast ball" sports: Do left-handers have an innate advantage? *British Journal of Psychology*, **80(2)**, 227–240.




KATAKAN KEPADAKU BAGAIMANA?
DUNIA PRASEJARAH
Bagaimana fosil ditemukan?
Bagaimana dinosaurus punah?
Bagaimana manusia mengalami evolusi?
Jawaban atas banyak pertanyaan


21-5
25
21-5
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Part I

Proximate factors underlying lateralization

2

Epigenesis of behavioural lateralization in humans and other animals

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Abstract

Despite several decades of research, the epigenesis of behavioural and brain lateralization is still elusive, although its knowledge is important in understanding developmental plasticity, function and evolution of lateralization, and its relationship with developmental disorders. Over the last decades, it has become clear that behavioural lateralization is not restricted to humans, but a fundamental principle in the organization of behaviour in vertebrates. This has opened the possibility of extending descriptive studies on human lateralization with descriptive and experimental studies on other vertebrate species. In this review, we therefore explore the evidence for the role of genes and environment on behavioural lateralization in humans and other animals. First, we discuss the predominant genetic models for human handedness, and conclude that their explanatory power alone is not sufficient, leaving, together with ambiguous results from adoption studies and selection experiments in animals, ample opportunity for a role of environmental factors. Next, we discuss the potential influence of such factors, including perinatal asymmetrical perception induced by asymmetrical head position or parental care, and social modulation, both in humans and other vertebrates, presenting some evidence from our own work on the domestic chick. We conclude that both perinatal asymmetrical perception and later social modulation are likely candidates in influencing the degree or strength of lateralization in both humans and other vertebrates. However, in most cases unequivocal evidence for this is lacking and we will point out further avenues for research.

Introduction

Lateralization of brain and behaviour refers to the fact that the hemispheres of the brain differentially control behaviour. It is also known as hemispheric or cerebral asymmetry/specialization (Vallortigara & Rogers 2005). At the behavioural level, it is often expressed in side biases for motor output, perception and information processing. For a long time, lateralization was considered unique to humans, but recently it has become clear that lateralization is a fundamental characteristic of the organization of brain and behaviour in vertebrates (Vallortigara & Rogers, 2005). Animal models open new and exciting perspectives for understanding the function and evolution and provide the opportunity to experimentally study the causes and consequences of lateralization.

It is highly likely that such a fundamental aspect of brain and behaviour is under the control of genetic encoding. However, this does not exclude an important role for environmental factors in the development and expression of lateralization. The debate whether behavioural and brain lateralization is caused by genetic or environmental factors has been long-standing (Annett 1978b; Laland *et al.* 1995; Provins 1997; Bishop 2001). Insight into the epigenesis of lateralization is highly relevant to understand both its evolution and possible constraints on plasticity as well as its adaptive flexibility and pathologies. By describing correlations between genetic information, environmental factors and the development or expression of lateralization, or by manipulating genetic and environmental factors using animal models, such insights can be acquired.

Especially in the psychological literature, there is some consensus about the genetic heritability of lateralization. This is mainly based on the distribution and genetic modelling of handedness in humans. Handedness is heritable as it runs in families. Only 7.6 per cent of the children of two right-handed parents are left-handed. This percentage increases to 19.5 per cent if one of the parents is left-handed and to 54.5 per cent if both the parents are left-handed (Rife 1940). Heritability estimates vary between 0.23 and 0.66 (Denny & O' Sullivan 2007). However, these data are no hard evidence for a genetic basis for the degree or direction of lateralization in itself. Traits may run in families owing to exposure to environmental factors that are more similar within than between families and other forms of non-genomic inheritance. Furthermore, heritability estimates can be influenced by these factors too, and can differ greatly depending on the environment in which the data were obtained.

In this paper, we review the evidence for genetic and environmental influences on brain and especially behavioural lateralization in humans and other animal species. We focus on handedness since this might be more sensitive to (especially postnatal) environmental factors than lateralization of cognitive functions. We will first discuss the explanatory power of the existing genetic models for human handedness, including their strengths and weaknesses followed by what is known of genetic influences on lateralization in other animal species. Next, we will focus on environmental influences and review evidence for humans and other vertebrate species. Section 4 summarizes and synthesizes both sections and offers suggestions for future research.

Explanatory power of genetic models

One of the most common ways to investigate lateralization in humans is measuring handedness in combination with cerebral dominance for speech for which several genetic theories have been proposed. We will briefly describe the features of the main genetic models and the findings that challenge the hypothesis that handedness is determined genetically. For each of these potential problems, we will explore to what extent environmental factors may be an alternative to the genetic explanation.

Models of genetic transmission of handedness

Although offspring of left-handed parents are more likely to be left-handed than offspring of right-handed parents, right-handed offspring can be produced by two left-handed parents (Rife 1940; McManus & Bryden 1992). The classical Mendelian approach incorporating a recessive allele for left-handedness (Jordan 1911) was therefore discarded. Subsequently, several other single-locus models were proposed. These models do not propose the existence of genes encoding for right-or left-handedness, but alleles for right-handedness (in combination with left-hemispheric dominance for language), and handedness (and language) becoming left-or right-lateralized by chance (Annett 1972, 1985, 2002; McManus 1985a, 1999; Klar 1996). This is to account for the finding that left-handers can be lateralized for language in either direction. In Annett's theory, a normal distribution (arising from environmental influences) of the difference in skill between the two hands exists. A 'right' allele, which encodes for left-cerebral dominance for speech, shifts this distribution to the right (increasing right-over left-hand skills; figure 1a). An individual with low left-hand skills and high right-hand skills is therefore likely to become right-handed (but not necessarily so if environmental factors, such as social pressures are high). By contrast, the right allele in McManus' and Klar's models encodes directly for right-hand preference and left-cerebral dominance (figure 1b,c, respectively). In these two models, homozygous individuals for the 'chance' allele (no right allele

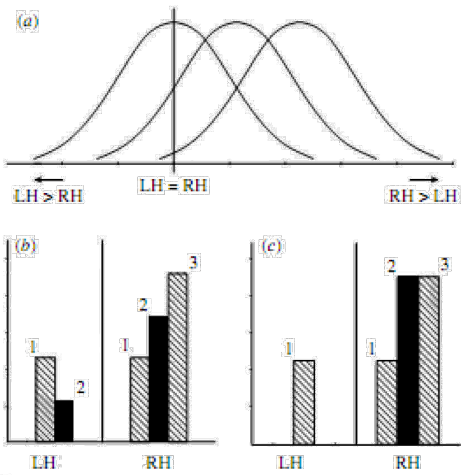


Figure 1 • Distribution of lateralization of handedness in proportion of individuals with a certain allele combination, according to three genetic models: (a) Annett 's model for hand skill, (b) McManus' model and (c) Klar's model, both for hand preference. 1, homozygote chance; 2, heterozygote; 3, homozygote for right allele. For details see text.

present) will be left-or right-handed with language left-or right-lateralized all with equal probabilities, whereas in Annett's model the skill distribution is centred around zero with approximately 50 per cent of these individuals better skilled with the right hand and 50 per cent with the left hand. Depending on the theory, heterozygotes become either right-handed (Klar 1996) or have an increased chance of becoming right-handed (Annett 1972, 1975; McManus 1985a, 1999). Homozygotes for the right allele will be right-handed according to McManus (1985a, 1999) and Klar (1996); inAnnett's model (1972, 1975), these individuals can still be lefthanders, owing to the fact that the model describes a shift in the distribution of skill between the hands that still extends, albeit at low frequency, into the better left-hand skilled range. In contrast to the single-locus models, Levy & Nagylaki (1972) proposed a two-loci, four-allele model. One locus encodes for cerebral dominance for speech, the other for either contralateral or ipsilateral hand control relative to the dominant hemisphere. Yeo & Gangestad (1993) proposed that there is little or no direct genetic effect on handedness. A deviation from the moderate right-handed population mean is assumed to be caused by early polygenetic homozygosity causing developmental instability and extreme right-or left-handedness.

Challenges for the models

(a) The twin paradox

There are several general problems concerning the validity of these genetic models. The first emerged from twin studies. To disentangle genetic from environmental factors, many investigators compared monozygotic (MZ) with dizygotic (DZ) twins. MZ twins are more likely to be concordant concerning handedness than DZ twins (for a meta-analysis see Sicotte *et al.* 1999), suggesting genetic inheritance. However, between 10 and 25 per cent of MZ twins are still discordant for handedness (Rife 1940; Bryden 1982; Sicotte *et al.* 1999). Several suggestions were made to fit this MZ twin discordance phenomenon into genetic models. Based on calculations concerning gene frequencies, Klar (1996) expected 18 per cent of the individuals in the population to be lacking the right gene and thus developing direction of lateralization by chance and this could explain the 18.3 per cent discordance in MZ twins found by Rife (1940). This is because lack of the fully penetrant right alleles in Klar's model would induce the individual members of MZ twins to develop handedness at chance independently of each other. However, just as many concordant as discordant twins with this genotype are expected on the basis of chance, so that 18 per cent of the genotype would lead to 9 per cent discordant and 9 per cent concordant twins. The theory can thus only account for half of the discordant MZ twins observed in the population. Furthermore, it cannot explain the higher incidence of left-handedness in twins compared with singletons (Sicotte *et al.* 1999). Similarly, both McManus (1985a, 1999) and Annett (1972, 1975) proposed that discordant MZ twins could be homozygotic for the chance allele. In addition, due to the additive nature of their models, discordant MZ twins can also be heterozygotic and in Annett's model even homozygotic for the right allele. An addition to Annett's (1978a) model assumes that the right shift caused by the right allele expresses weaker in those who are less mature at birth, and it was proposed that this is the case for twins relative to singletons. This decreased gene expression is assumed to be caused by disturbances of development during a sensitive prenatal period and would explain the high frequency of discordances and the increased incidence of

left-handedness in twins compared with singletons (Sicotte *et al.* 1999, but see Medland *et al.* 2003). However, Orlebeke *et al.* (1996) argued that decreased maturation and the supposedly associated reduced expression of the right shift cannot account for increased left-handedness in twins because the firstborn twin is heavier and still more often left-handed than the second-born twin. The model of Levy & Nagylaki (1972) states that individuals with identical genotypes exhibit the same dominant hemisphere for language and the same hand preference, and attributes the prevalence of discordant MZ twins to environmental factors such as pathogenic and mirror-imaging effects (see below).

(b) Explanations of the twin paradox by environmental factors

Clearly, solely genetic inheritance is unlikely to explain the twin paradox. Proposed environmental explanations for the high proportion of discordant MZ twins include the suggestion that the MZ twinning process itself is pathological (James 1983; Boklage 1987; Levin 1999; Sommer *et al.* 1999), and the mirror-imaging theory that states that owing to relatively late splitting of the already slightly lateralized embryo, the members of MZ twins represent the 'right' and 'left' halves of the egg (Newman 1928; Stocks 1933). However, the finding that the incidence of left-handedness is not different between MZ and DZ twins is in contrast with these two hypotheses (Sicotte *et al.* 1999). A more viable explanation is that discordant MZ twins are affected by differential environmental factors such as differential perinatal stress that is associated with higher incidences of left-handedness (Soper & Satz 1984; Sicotte *et al.* 1999 and references therein; Hopkins *et al.* 2000 for chimpanzees). For example, primiparae might be more exposed to birth stress (Orlebeke *et al.* 1996), twins might influence each other, and twin members lay in differential position in the womb (Geschwind & Galaburda 1985) possibly affecting lateralization in twins.

(c) Sex differences

The second challenge concerning the genetic models of handedness is that males show higher incidences of left-handedness than females (11.6% versus 8.6%; McManus 2002). A simple autosomal genetic theory may thus not explain this sex difference. Annett addressed the sex differences in handedness similarly to the way she addressed the twin paradox: the right allele would express weaker in those who are less mature at birth (Annett 1978a; Davis & Annett 1994), which in this case means less in males than females. The parameters of the model thus changes depending on the sex and singleton/twin state of the offspring. A revision of the McManus' model (1985a) incorporated a novel rare recessive allele located on the X chromosome, which suppresses the autosomal right allele (McManus & Bryden 1992). Higher incidences of left-handedness are then expected in males because males, having only one X chromosome, need only one of this rare recessive allele, whereas females need two. Several other sex-chromosomal linked models have been proposed (Crow 1993, 1995; Jones & Martin 2000). Laval *et al.* (1998) found evidence for a quantitative trait locus (QTL) on the X chromosome for linkage to relative hand skill. Although this was partly supported by a genome-wide scan, more important linkages to relative hand skill were found on other chromosomes (Francks *et al.* 2002). Another genome-wide analysis found no evidence for the presence of QTL linked to handedness on the X chromosome (Van Agtmael *et al.* 2003). These studies suggest that handedness has a genetic component, but that a single-gene model is unlikely and that the genetic factor influencing handedness is most probably multifactorial. However, it is conceivable

that these multiple genes may inherit as a single-locus trait. This is for example the case of co-adapted gene complexes that are linked due to their position on the same arm of an inversed part of a chromosome (Kamping & Van Delden 1999). In the case of genes being distributed over several chromosomes, inheritance as a single locus is, however, not conceivable. However, in that case it may account for the random factor postulated to determine lateralization, but not for the dominant allele that would induce right-handedness. Neurodevelopmental disorders are, just as left-handedness, more common in males. Yeo & Gangestad (1993) proposed that males show higher degrees of polygenetic homozygosity, inducing developmental instability leading to increased left-handedness. They however do not explain the cause of the supposedly increased homozygosity in males.

The incidence of left-handedness is higher when the mother is left-handed and the father is not, than when the father is left-handed and the mother is not (Falek 1959; Porac & Coren 1981; McManus 1991; Annett 1994; Mckeever 2000). This either suggests a form of genomic imprinting or parental effects. Annett addressed this problem based on a Carter (1961) effect. However, the Carter effect can occur when an inherited characteristic is genetically multifactorial, whereas Annett's model is not. As mentioned earlier, McManus & Bryden (1992) suggested an X-linked recessive gene that can suppress the autosomal right gene. This can explain not only the differences in incidences of left-handedness between males and females, but also this maternal effect. A female carrying two copies of this allele should then produce 100 per cent left-handed sons. Unfortunately, this prediction cannot be tested because the locus of this proposed gene is unknown, if it exists at all. Klar (1996) did not explain sex differences by genetic factors but attributes them and the maternal effects to environmental factors, such as differential sensitivity to social pressures (see below).

(d) Explanations of the sex difference and maternal effects by environmental factors

Several environmental factors may explain the higher incidence of left-handedness in males. First, men and women may differ in their sensitivity to social pressures. Females more often report to successfully change hand preference owing to social pressures. Furthermore, both males and females may be more under maternal than paternal social pressures, for example owing to more mother–offspring than father–offspring interactions (Morgan & Corballis 1978; Porac *et al*. 1986). In addition, as suggested by Falek (1959), left-handed fathers could also be more aware of the disadvantages concerning employment of left-handers than left-handed mothers. This could lead to higher social pressures when the father is left-handed than when the mother is. The offspring of left-handed fathers could thus more often conform to right-handedness. Additionally, it has frequently been suggested that sex differences in lateralization may be due to differential exposure to gonadal steroid hormones (reviewed in Pfannkuche *et al*. 2009).

(e) Inconsistencies with data

McManus (1985b) showed that a symmetrical bimodal model can describe the handedness skill distribution data at least for some tasks better than the right-shift model of Annett. The model of Klar also faces a problem. One of the predictions of Klar's (1996) model is that right-cerebral dominance for speech is expected in 50 per cent of left-

handed individuals (those lacking right alleles). However, several functional magnetic resonance imaging (fMRI) studies in non-pathological left-handers are in conflict with this (Jansen *et al*. 2007). Furthermore, the prediction that left-handed parents produce 50 per cent left-handed children does not hold (Annett 2008). The two-loci model of Levy & Nagylaki (1972) is inconsistent with the observation that left-handers tend towards ambilaterality, whereas right-handers show almost complete specialization of the hemispheres (Goodglass & Quadfasel 1954; Subirana 1964). If full expression of the alleles occurs only when a dominant allele is present at both loci, this problem is solved. This is however a post hoc addition to the model and should be tested in a new dataset.

Evidence from animal models

To validate the models and to disentangle between genetic and environmental factors influencing handedness, experimental studies should be performed. Owing to obvious ethical reasons, such studies can only be carried out in non-human animals.

(a) Descriptive evidence

In chimpanzees, handedness was measured by means of a tube task in which peanut butter must be obtained from a tube using one hand. Of the offspring of right-handed mothers 86 per cent were right-handed, but only in second to fifth offspring within a litter in which pregnancies have relatively low developmental instability. In the other offspring, only 46 per cent born to right-handed mothers were right-handed indicating both a heritable and environmental effect (Hopkins *et al*. 2001). In another study in wild chimpanzees, both maternal-offspring and maternal half-siblings hand preferences were significantly associated and concordance rates in mother–offspring and between maternal half-sibling were higher than chance (Lonsdorf & Hopkins 2005). Annett (2006) suggested that chimpanzees show a genetically determined right shift, although the magnitude of expression was significantly less than that in humans. Although her model may perhaps fit the data, this suggestion is in contrast with her idea that lateralization in handedness has evolved in consort with that for language, since chimpanzees lack the capacity for the latter. Alternatively, the heritable component can be explained by a non-genetical maternal effect (see p. 37).

(b) Experimental evidence

An attempt to selectively breed mice for the direction of pawedness failed, although selective breeding attempts for the degree of pawedness were successful (see Collins 1985 for a review). Variation within the latter strains was still present, suggesting environmental influences. Collins *et al*. (1993) showed that differences in total heterozygosity did not explain the difference in degree between the strains as was originally proposed by McManus (1992). We would like to point out that conclusions about the genetic background of a trait based on selective breeding experiments without cross-fostering the offspring should be made with caution. These experiments are not capable of distinguishing between genetic and environmental effects (such as learning). Moreover, in order to rule out any prenatal effects (such as hormones) on lateralization, zygote translocation is necessary. An artificial selection study in the poeciliid fish Girardinus falcatus on the preference to investigate certain stimuli with either the left or right eye estimated the

heritability of degree and direction greater than 0.5 (Bisazza *et al.* 2000). However, after the first generation, the response to selection ceased. Some potential explanations for the latter finding were suggested in which fish showing the lateralization opposite to the one selected for have an advantage. For example, fish of such opposite lateralization may surprise conspecifics in their approach from the other side, leading to more successful forced copulations or more successful predation (Bisazza *et al.* 2007). Hori (1993) investigated the inheritance pattern of lateralization in the fish Perissodus microlepis. These fish eat scales from the flanks of prey fish by attacking them from either the left or right side and have therefore a slightly asymmetrical mouth opening, directed to, respectively, the right or left. He suggested that this 'mouthedness' is inherited in a Mendelian fashion with right mouthedness being dominant. This is, however, not consistent with the finding that two left-mouthed parents can produce up to 25 per cent right-mouthed offspring. Later, Hori *et al.* (2007) adjusted the explanation by suggesting that the right-mouthed allele is lethal when homozygous. However, the data of Hori (1993) suggested that homozygous right-mouthed fish are present in the population. The inheritance pattern of this trait thus remains unclear.

In conclusion

Although several elegant genetic models for lateralization of handedness and language fit well the majority of the distribution and inheritance data by assuming certain rules for genetic inheritance, they require several ad hoc additions for explaining deviations from the main pattern. These additions are not always fully supported by independent data. This may either suggest that the specific deviations, such as the twin, sex and maternal effects, may be best explained by environmental factors, for which indeed some suggestions have been made in the literature; or it may even suggest that the basic assumptions of the models are not correct, as has been discussed earlier. The latter is supported by the fact that the few genome scans performed concerning handedness could not find evidence for a simple genetic model, but suggest a more complex interplay between different genes involved. In any case, the models do not rule out an important role for environmental influences on the development of lateralization. Interestingly, models such as those from Annett (1972, 1985) and Klar (1996) explicitly need environmental factors to fit the observed incidences of left-handedness. Few attempts to identify the potential genetic background of handedness in non-human animals have been performed. So far, the results are inconsistent with each other and with the human models proposed, although Annett (2006) suggested some resemblance between humans and chimpanzees in the genetic inheritance of hand-use lateralization. No genetic models for lateralization in animals have been built and human models have hardly been tested in animals. More animal studies are crucially needed to investigate the inheritance of laterality in animals. This could shed light on its evolution and generate hypotheses for its inheritance in humans.

Potential environmental factors

Section 2 indicates that there is ample opportunity for environmental factors to affect the development of behavioural lateralization. In this section, we will discuss these factors in more detail, focusing on the potential effects of social modulation such as social pressures and parental effects (including cradling), as well as asymmetric input of stimuli. Additionally, the organizational and activational effects of steroid hormones have been suggested to be relevant for lateralization. This topic will be discussed, together with sex differences in lateralization, in a separate paper where we present the results of several meta-analyses (Pfannkuche *et al.* 2009). We start with a short description of development of behavioural lateralization in order to establish when, and to what extent, it gradually develops. This may indicate to what degree and in which stage in development there is scope for environmental effects to act. We will not focus on pathological development. It is well known that the incidence of left-handedness is positively related to behavioural disorders, birth stress and low birth weight (Bakan *et al.* 1973; Coren 1993 for a review), and this is reviewed in another contribution to this issue (Llaurens *et al.* 2009).

Early development of handedness

Human foetuses prefer to use the right hand for thumb sucking already in the third trimester independent of lying position in the womb (Hepper *et al.* 1991). Thumb-sucking behaviour, but no other prenatal hand–mouth contacts (de Vries *et al.* 2001), is a good predictor for handedness later in life (Hepper *et al.* 2005). Similarly, prenatal head position shortly before birth correlates with the preferred head position of neonates in a supine position, which again correlates with handedness in reaching tasks 12–74 weeks postpartum (see p. 35). Although these data suggest that predispositions for handedness are already present early in ontogeny, they do not exclude a role for environmental factors affecting lateralization later in life. In fact, prenatal influences may be very important (see below and e.g. Pfannkuche *et al.* 2009). In addition, during early childhood, handedness still shows considerable fluctuations (Gesell & Ames 1947; Goldfield & Michel 1986; Corbetta *et al.* 2006; Michel *et al.* 2006). Not until the age of 4 years right-handed behaviour predominates and unilateral hand preference is well established at the age of 9 (Gesell & Ames 1947). Therefore, the data suggest that although predispositions for lateralization are present already early in ontogeny, handedness is still open to environmental influences later in life, much as early predispositions for motor patterns (courtship postures and calls) and cognition (imprinting on the mother) in birds can still be modified in later life (Johnson *et al.* 1985; Groothuis 1993).

Environmental factors: asymmetric input of stimuli

(a) Head position in humans

A few weeks before birth, the foetus' head position becomes fixed in utero. Of the 97 per cent of foetuses that lie in a cephalic position, two-thirds lie with their right ear and one-third with the left ear facing out (Michel & Goodwin 1979; Previc 1991 and references therein). This position correlates strongly with the head position of the neonates that lie in a supine position (Michel & Goodwin 1979). The supine head orientation affects the

experience with the right and left hand. Previc (1991) has argued that this 2 : 1 ratio is more characteristic for many behavioural asymmetries in human and non-human populations than the 9 : 1 ratio typical for human handedness. He proposes that these asymmetries originate from an asymmetrical prenatal development of the ear and labyrinth. Speech is then lateralized through a slight right ear advantage in the mid-frequency sound range. This advantage is derived from an asymmetrical craniofacial development. Vestibular lateralization, which is linked to motor behaviour, can be traced back to the asymmetrical head position of the foetus during the final trimester. This asymmetry would come about through the differential experience of the left and right vestibules in the final trimester caused by motoric movements of the mother, perhaps creating a pathway for maternal effects discussed earlier. Most (70–80%) neonates prefer to turn their head to the right side when they are in a supine position (Michel & Goodwin 1979; Michel 1981; Konishi *et al*. 1986; Previc 1991; Ronnqvist *et al*. 1998; Ronnqvist & Hopkins 2000; Damerose & Vauclair 2002). This preference appears at the second day of life, at which time they are also more reactive to sounds on the right-hand side (Turkewitz *et al*. 1966). This tendency diminishes in the course of development. The supposed effects on functional motor lateralities have therefore been argued to be only transient (Konishi *et al*. 1986). However, the amount of spontaneous visual experience with each hand, which is dominated by head position, predicts which hand predominated in visually elicited reaching at 12 weeks (Coryell & Michel 1978). Moreover, as already mentioned, head orientation in a supine position correlates with handedness during reaching in the period 12–74 weeks postpartum (Kuo & Shen 1937). Inducing differential experience with hands during early development has been a worldwide natural experiment. Across the globe, there have been large-scale changes in placing babies in a supine or prone position in their cribs, due to change in medical advice. As mentioned, in the supine position, there is a natural bias towards right-hand use, whereas in the prone position there is no expression of preference. This is because of the parental strategy of alternating the baby's head to the left and right in order to avoid asymmetrical skull development, and because of the baby's inability to change the head position in the first months by itself. Interestingly, there was an increase in non-right-handed toddlers (at 18 months of age) that were reared in the prone position (Konishi *et al*. 1987). This suggests that head position is causative to handedness. We are currently conducting a study in The Netherlands to see whether we can replicate this finding.

(b) Head position in other animal species

Except for birds, it is unknown whether head position is related to lateralization of brain and behaviour in nonhuman species. Owing to the asymmetrical position of the avian head in the egg, one eye is positioned against the body, whereas the other lies against the eggshell. Light can penetrate the shell and induce brain lateralization (see below). However, one should realize that the indirect effect of head position on lateralization via its effect on light input has not been disentangled from a direct effect of head position, irrespective of light exposure. Although avian models are often used to study the development of lateralization, quantitative data support the general idea that bird embryos are folded in the egg in such a way that almost all of them receive light with the right eye due to their head position (Oppenheim 1973) are surprisingly scarce, and

some data suggest much more variation (Riedstra 2003). If the variation of head position is substantial, this may severely influence the outcome of experiments manipulating embryonic light exposure. We found that fMRI techniques can be successfully used to identify the turning position in eggs without exposing them to light (B. Riedstra 2007, personal observation).

(c) Asymmetric light input in birds

Many bird species (galliformes, pigeons, parrots, raptors and songbirds) show behavioural lateralization in visually guided behaviours (e.g. Andrew & Brennan 1983; ten Cate *et al*. 1990; ten Cate 1991; Rogers 1996; Alonso 1998; Manns & Gunturkun 1999; Bobbo *et al*. 2002; Templeton & Gonzalez 2004), motor patterns (Rogers & Workman 1993; Goller & Suthers 1995; Csermely 2004) and cognitive functions (Nottebohm 1970, 1971; Clayton & Krebs 1994, 1995; Floody & Arnold 1997; Gagliardo *et al*. 2001; Nottelmann *et al*. 2002). Lateralization of visually guided behaviours is influenced by asymmetrical light exposure in the period shortly before hatching. Light reaching the eye through the eggshell induces growth of the visual projections from the exposed eye to the contralateral hemisphere and induces functional lateralization (Rogers 1996). Hemispheric control of attack and copulation becomes dominant in the hemisphere contralateral of the light-exposed eye, both when exposing the naturally exposed eye or by experimentally exposing the normally occluded eye to light (Rogers 1990). Chicks receiving no light also become lateralized but the direction of lateralization is unpredictable (Rogers 1982). In addition, dark-incubated chicks become less strongly lateralized and have poorer performances in dual tasks (Dharmaretnam & Rogers 2005). Unfortunately, further studies addressing the extent and nature of lateralization in dark-incubated chicks are lacking, although these could reveal to what extent other factors than light guide the development of lateralization. It is not our intention here to review the literature on light-induced lateralization in birds since excellent reviews on this topic are available (e.g. Rogers 1996). However, we stress that there is no evidence showing that asymmetrical light exposure during the last phase of incubation is really the default situation in nature. Only one study detailed the amount of light exposure to eggs during the incubation period and concluded that this was sufficient to induce lateralization (Buschmann *et al*. 2006). As there is large variation in eggshell properties, nest sites determining light availability and incubation patterns among avian species, the generality is questionable. Moreover, the adaptive advantage of lateralization has recently been questioned too (Hirnstein *et al*. 2008). In addition, only one study has addressed the question of whether manipulation of light exposure during incubation has consequences in adulthood (Manns & Gunturkun 1999). This is very relevant as the effect of early light exposure on asymmetrical visual pathways seem to diminish with age in the chicken (Rogers 1995). Since we are here concerned with the mechanisms of development of lateralization, and not its functional relevance, this will not be a topic of this paper. Finally, light has pleiotropic effects that may confound experiments that manipulate embryonic light exposure. Prenatal light exposure also increases growth rate and hatching time but reduces hatchling weight (Adam & Dimond 1971; Evans & Evans 1999; Shafey & Al-Mohsen 2002; Shafey 2004). If these factors affect behavioural and brain lateralization, as birth weight and perinatal stress in humans, then light may affect lateralization via other pathways than asymmetrical light input only.

(d) Cradling in humans

Right-handed and dextro-cordius mothers prefer to hold infants on the left arm (left-handed females have not been reported for right-side-holding biases, but no sufficient data exist; Donnot 2007), whereas males have no preference (Damerose & Vauclair 2002). Cradling by mothers thus induces asymmetrical auditive and visual input, head and arm position, potentially influencing development of lateralization. However, left-handed cradling may actually restrict right-arm movements of the baby and thereby perhaps development of right-handedness. Furthermore, although there is some evidence that the emotional hemispheric specialization of the holder predicts holding bias in left-handed students, but not in left-handed mothers (Donnot 2007), the effect on the baby's lateralization is not yet known. There is also some evidence that the baby's head-turning preference modulates the side preference of adult handling, but not the other way around (Bundy 1979). In conclusion, evidence for an influence on lateralization of the baby is lacking. Longitudinal studies on children until their hand preference are stable in relation to cradling experience, for example in societies that differ in cradling behaviour, may be of help. This may perhaps also explain part of the difference in the frequency of left-handedness observed among societies (see also p. 38).

Environmental factors: adoption in humans and animals

In an attempt to disentangle between genetic and environmental factors determining handedness, investigators have focused on adoption studies. Surprisingly, parent–offspring correlations concerning strength and direction of hand preference were absent in both adopted and non-adopted children (Rice *et al.* 1984), perhaps due to the very young age of the children investigated (12–24 months). Two other studies showed different results. Hicks & Kinsbourne (1976) found that hand preferences of students significantly correlated with the writing hand of their biological parent, but not with that of their step-parent. Although the authors statistically controlled for the time spent living with the step-parent, it is most likely that the hand preference was already established in the students long before the step-parent could influence this preference, since the mean age of the students when the step-parent moved in was approximately 13 years of age (s.d.=3.12). However, a similar outcome was found in a study in which all adopted children were taken into the participating families before the age of 1 (Carter-Saltzman 1980). However, the possibility that lateralization and handedness are determined before that age, although not yet fully expressed, is still conceivable (see pp. 28, 34-35, 37; Pfannkuche *et al.* 2009). To our knowledge, only one cross-fostering study on handedness, measured by means of a tube task with peanut butter (see above), has been conducted in non-human animals. In cross-fostered chimpanzee siblings, the concordance rate in hand preference was not greater than chance, whereas this was the case for siblings that were reared together, strongly suggesting that the underlying mechanisms controlling handedness are heritable, but not genetic (Hopkins 1999). In conclusion, early cross-fostering studies suggest a strong heritable component, and the chimpanzee studies indicate that this may be a non-genetic effect.

Changes with age

In humans, cross-sectional studies reveal that right-handedness increases with age (Fleminger *et al.* 1977; Smart *et al.* 1980; Brackenridge 1981; Brito *et al.* 1985; Beukelaar & Kroonenberg 1986; Lansky *et al.* 1988; Dellatolas *et al.* 1991; Gilbert & Wysocki 1992; Iwasaki *et al.* 1995; De Agostini *et al.* 1997; Ellis *et al.* 1998; McManus 2002). Several hypotheses have been postulated to explain this phenomenon.

(i) Since left-handedness has been correlated to lower survival, this might result in the decrease in the incidence of left-handedness among elderly people (Halpern & Coren 1988; Coren 1989; Coren & Halpern 1991).

(ii) Social pressures against left-handedness over the years declined, so that younger people are less restricted and therefore show higher incidences of left-handedness (Hildreth 1949; Levy1974;Brackenridge1981; Leiber& Axelrod 1981 and references in Harris 1990). Furthermore, with increasing age, the number of social contacts increase, which may enhance the probability to switch towards right-handedness.

(iii) Humans live in a right-biased world. Tools are made for right-handed individuals and this will in time cause a shift towards dextrality in left-handed individuals and strengthens right-handedness in right-handers (Porac & Coren 1981).

(iv) Cerebral dominance development is a continuous process that evolves throughout life and causes the increase in right-handedness with age (Brown & Jaffe 1975; Fleminger *et al.* 1977).

(v) An information bias in handedness questionnaires has been proposed, resulting in a change in the categorization of handedness (Fleminger *et al.* 1977). This does not seem likely as most studies investigating the effect of age on handedness are cohort studies. To distinguish between these hypotheses, longitudinal studies that investigate the development of lateralization within the individuals are clearly needed.

Environmental factors: social pressures

(a) Evidence in humans

Although right-handers outnumber left-handers in all societies studied, differences in the percentages of right-handedness have been observed among different societies: sinistrality being, in general, higher in Western societies than in other societies (Iwasaki 2000 and references therein). These differences could be caused either by environmental factors such as increased social pressures in some societies, or by a decreased number of the proposed right allele in the gene pool of certain populations. McManus (2002) hypothesized that it was possible to disentangle between these genetic and environmental factors by investigating how strongly handedness runs in families. He assumed that if social pressures to be right-handed are strong, left-handedness will run less strongly in families. This assumption is not necessarily right as differences in social pressures may not be equal for all individuals and vary between families. Porac *et al.* (1986) found some evidence for this. She investigated social pressures within families by assessing the amount of attempts to switch handedness: males from right-handed parents were more likely to switch from left-to right-hand use than males from one or two left-handed

parents. McManus' conclusion that the decreased incidence of left-handedness in non-Western populations is due to a decreased incidence of the right allele might be false as it can also be explained by differential social pressures between families. The hypothesis that social pressures can decrease the incidence of left-handedness is further strengthened by the finding of Dawson (1977) who found that more conforming agriculturalists measured by means of the Asch Conformity Test show low incidences of left-handedness (0.6–3.4%), whereas permissive, nonconforming populations show extensively higher incidences of left-handedness (11.3–10.5%).

(b) Evidence for other animal species

To our knowledge, there are no studies that have tested the possibility of social modulation affecting behavioural lateralization in non-human animals. However, we recently found some evidence for this possibility. Eggs of laying hens (*Gallus gallus domesticus*) were incubated under standard conditions. The chicks were housed in 10 groups of 6 (± 1) individuals in the same room. At day 4–6, post-hatching behavioural lateralization was assessed by detour tests. Chicks had to detour a see-through barrier to reach either a group of unknown conspecifics or a mealworm. The side used to detour the barrier was scored in two bouts of five consecutive trials on two consecutive days for both stimuli. Preferred eye use for each stimulus, determined by hemispheric organization, is thought to determine the side of rounding the barrier (Vallortigara *et al.* 1999). Individuals showed consistent choices between tests (r^2 =0.69, p<0.0001, n=58), with most individuals preferring to turn right in both tests. This suggests that approaching food and unknown conspecifics are functionally located in the same, predominantly right hemisphere. Interestingly, the variation in lateralization was smaller within groups than among groups (figure 2; F=12.66, p<0.001). This is the first evidence suggesting that lateralization of visually guided behaviours can be modulated by post-hatching social interactions. This could ensure the hypothesized benefits of a group bias in lateralization, as suggested by Vallortigara & Rogers (2005). However, since the experiment was not designed for testing this hypothesis, this post hoc finding is currently being replicated. Furthermore, Collins (1968) conducted an experiment in which the effects of social pressures were altered. He found that in a non-biased environment (no pressure) in which

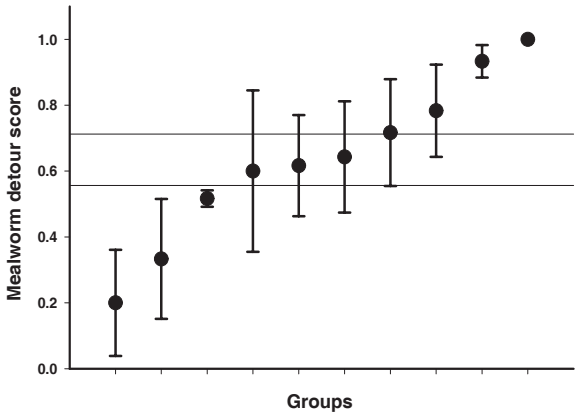


Figure 2 • Detour scores: each circle represents the group mean (±s.e.) of six chicks, which round a barrier to reach a mealworm. Variation in lateralization was smaller within than between groups (F=12.66, p<0.001) indicating that lateralization was modulated by post-hatching social interactions.

food could be obtained with either paw equally well, mice had a side preference, but no population bias was observed. When the feeding tube was placed against the right wall in such a way that obtaining food was easier using the right than the left paw (mimicking the right-biased world of humans), 90 per cent of the mice showed a right paw preference (Collins 1975). This result was attributed to a change in paw use in weakly left-lateralized individuals. If this is the case, the direction and degree of handedness are not independent factors. Collins suggested that right-handedness might work similarly in humans. Collins *et al.* (1993) concluded that the observed differences found in the heritability between degree and direction in humans and mice should not necessarily lead to different underlying mechanisms. In conclusion, evidence exists for social modulation of handedness in both humans and other animal species.

Discussion

The long-standing debate about the question of whether lateralization of brain and behaviour is caused by genes or environment actually focuses on a wrong question. Modern developmental biology has recognized for decades that the phenotype develops under the continuous interaction between genetic and environmental influences and that both are indispensable for development. Moreover, in the end product of this interactive developmental process, both factors are impossible to disentangle. Therefore, by demonstrating the influence of either genetic or environmental components, we cannot conclude anything conclusive about the contribution of the other component on the developmental process. However, correlative and experimental studies can demonstrate which factors are important, and how they interact. Unfortunately, gene–environment interactions have not been explicitly studied, but for instance the difference in lateralization between light-and dark-reared birds in which the latter still show some degree of it (Rogers 1995) does suggest such an interaction. Moreover, owing to the historical focus on humans, descriptive studies outnumber experimental studies by far. We hope that this review will stimulate researchers to bring the field more into balance. It has been questioned to what extent lateralization in humans and other vertebrates may be comparable. We agree that it is likely that humans may have species-specific adaptations in their lateralized behaviour. This may explain the strong human lateralization in handedness due to selection on efficient tool use or language (Corballis 2003). Nevertheless, we strongly believe that lateralization of brain and behaviour, being such a fundamental aspect of the organization in vertebrates, must share common principles for humans and other vertebrates, similarly to the blueprint for vertebrate skeleton, physiology, brain and behaviour. Evidence for a genetic basis of lateralization in humans is mainly based on demographic and heritability studies of handedness, and the explanatory power of genetic models. As argued earlier, the evidence from demographic and heritability studies does not disentangle genetic from environmental factors such as parental effects, and even early adoption studies cannot circumvent prenatal maternal effects. Evidence from the modelling approach is not yet fully convincing either. Despite their elegance and clever design, the models have limited explanatory power and are not backed up by the data from human genome scans, which suggest a multi-genetic control of human lateralization. Unfortunately, data from animal experiments concerning the genetics of

lateralization are also inconclusive. Although the few selection experiments in animals give some support for genomic heritability, the results are ambiguous and the studies not always properly conducted. Although some data suggest exciting possibilities, evidence for environmental influences on lateralization is ambiguous too. Descriptive data that show changes with age are not conclusive for environmental effects since they may be genetically encoded. Moreover, in order to assess developmental principles of lateralization, longitudinal studies are needed. In humans, the available data suggest that although predispositions for handedness may be present already prenatally and predictive for later lateralization, handedness can to some extend still change in later life. The correlation between early developmental disorders and left-handedness suggests a role for early environmental modulation, but does not tell us necessarily much about the environmental effects on undisturbed development. The possibility that in humans, left-handers are in fact a heterogeneous group of pathological and 'normal' left-handers complicates research to a large extent. Actually, the genetic models suggest that also the right-handers are a heterogeneous group consisting of both genetically right-and left-handers. Interpretation is further complicated by the use of different criteria to categorize handedness. Finally, more attention should be paid to other forms of behavioural lateralization, which may not always correlate with handedness, and may be more similar to lateralization indices in animals. Unfortunately, in animals even less is known about typical development and to what extent early manipulations still exert their effect in adulthood. Such long-term studies take time, but are very relevant for further progress in the field. Five lines of evidence suggest a role for environmental modulation of lateralized behaviour. First, the finding that rearing position of the neonate seems to affect handedness, based on a natural experiment whereby mothers were instructed differently to keep their babies in a supine or prone position (Konishi *et al.* 1987). It opens an exciting perspective, although we cannot rule out a confounding effect of time here and the study needs replication. Second, the study of cross-fostered chimpanzees (Hopkins 1999) indicated strong rearing effects, although this is in contrast with a study of early cross-fostering in humans (Carter-Saltzman 1980). Third, there is evidence that prenatal exposure to steroid hormones affects lateralization in humans (Pfannkuche *et al.* 2009). Fourth, our data on social modulation in the domestic chick warrant further research in this direction. Fifth, the effect of asymmetrical light input caused by the asymmetrical position of the head in bird embryos has now become a classical example of how early environmental factors can influence lateralization. This is consistent with the suggestion that pre-and post-natal head position may affect lateralization by asymmetrical perception in humans. Nevertheless, further studies documenting head position and light input in bird eggs and their long-term effects are necessary for interpreting the findings from a functional perspective. Furthermore, by manipulating head position together with light input, the influence of both factors can be disentangled.

In conclusion, there is evidence for both genes and environment to affect the development of behavioural lateralization, but evidence for both and especially their interaction is surprisingly incomplete. With the identification of the human genome, and the use of animal models, we believe that substantial progress can be made in the near future. For example, by setting up selection lines for differences in strength or direction in lateralization and exposing them to different environmental influences such as prenatal hormone

exposure, asymmetrical stimulus input, or exposure to conspecifics that are lateralized in only one direction, gene–environment interactions can be studied experimentally.

All experiments were carried out under license of the animal experiments committee of the University of Groningen (DECnr 4519).

Acknowledgements

We thank Reint Geuze and two anonymous referees for their helpful suggestions that improved the manuscript, and Mirre Simons and Gert Stulp for their help with the chick experiment on social modulation. The paper was written with the help of an EU grant (EDCBNL network) to TGGG.

References

Adam, J. H. & Dimond, S. J. 1971. Influence of light on the time of hatching in the domestic chick. *Animal Behaviour*, **19**, 226–229.

Alonso, Y. 1998. Lateralization of visual guided behaviour during feeding in the zebra finches (*Taeniopygia guttata*). *Behavioural Processes*, **43**, 257–263.

Andrew, R. J. & Brennan, A. 1983. The lateralization of fear behaviour in the male domestic chick: a developmental study. *Animal Behaviour* **31**, 1166–1167.

Annett, M. 1972. The distribution of manual asymmetry. Br. J. Psychol. 63, 343–358. Annett, M. 1975 Hand preference and the laterality of cerebral speech. *Cortex* **11**, 305–328.

Annett, M. 1978a. A single gene explanation of right and left handedness and brainedness. Coventry, UK: Lanchester Polytechnic.

Annett, M. 1978b. Genetic and non-genetic influences on handedness. *Behavior Genetics*, **8**, 227–249.

Annett, M. 1985. Left, right, hand and brain: the right shift theory. London, UK: Erlbaum.

Annett, M. 1994. Handedness as a continuous variable with dextral shift: sex, generation, and family handedness in subgroups of left-handers and right-handers. *Behavior Genetics*, **24**, 51–63.

Annett, M. 2002. Handedness and brain asymmetry: the right shift theory. Hove, UK: Psychology Press.

Annett, M. 2006. The distribution of handedness in chimpanzees: estimating right shift in Hopkins' sample. *Laterality*, **11**, 101–109.

Annett, M. 2008. Tests of the right shift genetic model for two new samples of family handedness and for the data of McKeever (2000). *Laterality*, **13**, 105–123.

Bakan, P., Dibb, G. & Reed, P. 1973. Handedness and birth stress. *Neuropsychologia*, **11**, 363–366.

Beukelaar, L. J. & Kroonenberg, P. M. 1986. Changes over time in the relationship between hand preference and writing hand among left-handers. *Neuropsychologia*, **24**, 301–303.

Bisazza, A., Facchin, L. & Vallortigara, G. 2000. Heritability of lateralization in fish: concordance of right-left asymmetry between parents and offspring. *Neuropsychologia*, **38**, 907–912.

Bisazza, A., Dadda, M., Facchin, L. & Vigo, F. 2007. Artificial selection on laterality in the teleost fish Girardinus falcatus. *Behavioural Brain Research*, **178**, 29–38.

Bishop, D. V. M. 2001. Individual differences in handedness and specific speech and language impairment: evidence against a genetic link. *Behavior Genetics*, **31**, 339–351.

Bobbo, D., Galvani, F., Macetti, G. G. & Vallortigara, G. 2002. Light exposure of the chick embryo influences monocular sleep. *Behavioural Brain Research*, **134**, 447–466.

Boklage, C. E. 1987. Twinning, nonrighthandedness, and fusion malformations: evidence for heritable causal elements held in common. *American Journal of Medical Genetics B*, **28**, 67–84.

Brackenridge, C. J. 1981. Secular variation in handedness over 90 years. *Neuropsychologia*, **19**, 459–462.

Brito, G. N. O., Brito, L. S. O. & Paumgartten, F. J. R. 1985. Effect of age on handedness in Brazilian adults is sex-dependent. *Perceptual Motor Skills*, **61**, 829–830.

Brown, J. W. & Jaffe, J. 1975. Hypothesis on cerebral dominance. *Neuropsychologia* **13**, 107–110.

Bryden, M. P. 1982. Laterality, functional asymmetry in the intact brain. New York, NY: Academic Press.

Bundy, R. S. 1979. Effects of infant head position on sides preference in adult handling. *Infant Behavior and Development*, **2**, 355–358.

Buschmann, J. U. F., Manns, M. & Gunturkun, O. 2006. "Let there be light!" Pigeon eggs are regularly exposed to light during breeding. *Behavioural Processes*, **73**, 62–67.

Carter, C. O. 1961. The inheritance of congenital pyloric stenosis. *British Medical Bulletin*, **17**, 251–253.

Carter-Saltzman, L. 1980. Biological and sociocultural effects on handedness: comparison between biological and adoptive families. *Science*, **209**, 1263–1265.

Clayton, N. S. & Krebs, J. R. 1994. Memory for spatial and object related cues in food-storing and nonstoring birds. *Journal of Comparative Physiology A* , **174**, 371–379.

Clayton, N. S. & Krebs, J. R. 1995. Lateralization in memory and the avian hippocampus in food-storing birds. In E. Alleva, A. Fasolo, H.-P. Lipp, L. Nadel & L. Ricceri, eds. *Behavioural brain research in naturalistic and semi-naturalistic settings*, pp. 139–157. Dordrecht, The Netherlands: Kluwer Academic.

Collins, R. L. 1968. On inheritance of handedness. I. Laterality in inbred mice. *Journal of Heredity*, **59**, 9–12.

Collins, R. L. 1975. When left-handed mice live in right-handed worlds. *Science*, **187**, 181–184.

Collins, R. L. 1985. On the inheritance of direction and degree of asymmetry. In S. Glick , ed. *Cerebral lateralization in nonhuman species*, pp. 41–71. Orlando, FL: Academic Press.

Collins, R. L., Sargent, E. E. & Neumann, P. E. 1993. Genetic and behavioral tests of the McManus hypothesis relating response to selection for lateralization of handedness in mice to degree of heterozygosity. *Behavior Genetics*, **23**, 413–421.

Corballis, M. C. 2003. From mouth to hand: gesture, speech, and the evolution of right-handedness. *Behavioral and Brain Sciences*, **26**, 199–260.

Corbetta, D., Williams, J. & Snapp-Childs, W. 2006. Plasticity in the development of handedness: evidence from normal development and early asymmetric brain injury. *Developmental Psychobiology*, **48**, 460–471.

Coren, S. 1989. Left-handedness and accident-related injury risk. *American Journal of Public Health*, **79**, 1040–1041.

Coren, S. 1993. The left-hander syndrome: the causes and consequences of left-handedness. New York, NY: Vintage Books.

Coren, S. & Halpern, D. F. 1991. Left-handedness: a marker for decreased survival fitness. *Psychological Bulletin*, **109**, 90–106.

Coryell, J. & Michel, G. F. 1978. How supine postural preferences of infants can contribute towards the development of handedness. *Infant Behavior and Development*, **1**, 245–257.

Crow, T. J. 1993. Sexual selection, Machiavellian intelligence, and the origins of psychosis. *Lancet*, **342**, 594–598.

Crow, T. J. 1995. A Darwinian approach to the origins of psychosis. *British Journal of Psychiatry*, **167**, 12–25.

Csermely, D. 2004. Lateralisation in birds of prey: adaptive and phylogenetic considerations. *Behavioural Processes*, **67**, 511–520.

Damerose, E. & Vauclair, J. 2002. Posture and laterality in human and non-human primates: asymmetries in maternal handling and the infant's early motor asymmetries. In L. J. Rogers & M. Andrew, eds. *Comparative vertebrate lateralization*, pp. 306–362. Cambridge, UK: Cambridge University Press.

Davis, A. & Annett, M. 1994. Handedness as a function of twinning, age and sex. *Cortex*, **30**, 105–111.

Dawson, J. L. M. B. 1977. An anthropological perspective on the evolution and lateralization of the brain. *Annals of the New York Academy of Sciences*, **299**, 424–447.

De Agostini, M., Khamis, A. H., Ahui, A. M. & Dellatolas, G. 1997. Environmental influences in hand preference: an African point of view. *Brain Cognition*, **35**, 151–167.

Dellatolas, G., Tubert, P., Castresana, A., Mesbah, M., Giallonardo, T., Lazaratou, H. & Lellouch, J. 1991. Age and cohort effects in adult handedness. *Neuropsychologia*, **29**, 225–261.

Denny, K. & O'Sullivan, V. 2007. The economic consequences of being left-handed: some sinister results. *Journal of Human Resources*, **42**, 353–374.

de Vries, J. I. P., Wimmers, R. H., Ververs, I. A. P., Hopkins, B., Savelsbergh, G. J. P. & Van Geijn, H. P. 2001. Fetal handedness and head position preference: a developmental study. *Developmental Psychobiology*, **39**, 171–178.

Dharmaretnam, M. & Rogers, L. J. 2005. Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research*, **162**, 62–70.

Donnot, J. 2007. Lateralisation of emotion predicts infant-holding bias in left-handed students, but not in left-handed mothers. *Laterality*, **12**, 216–217.

Ellis, S. J., Ellis, P. J., Marshall, E., Windridge, C. & Jones, S. 1998. Is forced dextrality an explanation for the fall in the prevalence of sinistrality with age? A study in northern England. *Journal of Epidemiology and Community Health*, **52**, 41–44.

Evans, C. S. & Evans, L. 1999. Chicken food calls are functionally referential. *Animal Behaviour*, **58**, 307–319.

Falek, A. 1959. Handedness: a family study. *American Journal of Human Genetics*, **11**, 52–62.

Fleminger, J. J., Dalton, R. & Standage, K. F. 1977. Age as a factor in handedness of adults. *Neuropsychologia*, **15**, 471–473.

Floody, O. R. & Arnold, A. P. 1997. Song lateralization in the zebra finch. *Hormones and Behavior*, **31**, 25–34.

Francks, C., Fisher, S. E., MacPhie, I. L., Richardson, A. J., Marlow, A. J., Stein, J. F. & Monaco, A. P. 2002. A genomewide linkage screen for relative hand skill in sibling pairs. *American Journal of Human Genetics*, **70**, 800–805.

Gagliardo, A., Ioale, P., Odetti, F., Bingman, V. P., Siegel, J. J. & Vallortigara, G. 2001. Hippocampus and homing in pigeons: left and right hemispheric differences in navigational map learning. *European Journal of Neuroscience*, **13**, 1617–1624.

Geschwind, N. & Galaburda, A. M. 1985. Cerebral lateralization: biological mechanisms, associations, and pathology. I. A hypothesis and a program for research. *Archives of Neurology*, **42**, 428–459.

Gesell, A. & Ames, L. B. 1947. The development of handedness. *Journal of Genetic Psychology*, **70**, 155–175.

Gilbert, A. N. & Wysocki, C. J. 1992. Hand preference and age in the United States. *Neuropsychologia*, **30**, 601–608.

Goldfield, E. C. & Michel, G. F. 1986. The ontogeny of infant bimanual reaching during the first year. *Infant Behavior and Development*, **9**, 81–89.

Goller, F. & Suthers, R. A. 1995. Implications for lateralization of bird song from unilateral gating of bilateral motor-patterns. *Nature*, **373**,63–66.

Goodglass, H. & Quadfasel, F. A. 1954. Language laterality in lefthanded aphasics. *Brain*, **77**, 521–548.

Groothuis, T. G. G. 1993. The ontogeny of social displays: form development, form fixation, and change in context. *Advances in the Study of Behavior*, **22**, 269–322.

Halpern, D. F. & Coren, S. 1988. Do right-handers live longer? *Nature*, **333**, 213.

Harris, L. J. 1990. Cultural influences on handedness: historical and contemporary theory and evidence. In S. Coren, ed. *Left-handedness: behavioral implications and anomalies*, pp. 195–258. Amsterdam, The Netherlands: Elsevier.

Hepper, P. G., Shahidullah, S. & White, R. 1991. Handedness in the human fetus. *Neuropsychologia*, **29**, 1107–1111.

Hepper, P. G., Wells, D. L. & Lynch, C. 2005. Prenatal thumb sucking is related to postnatal handedness. *Neuropsychologia*, **43**, 313–315.

Hicks, R. E. & Kinsbourne, M. 1976. Human handedness: partial cross-fostering study. *Science*, **192**, 908–910.

Hildreth, G. 1949. The development and training of hand dominance. I. Characteristics of handedness. *Journal of Genetic Psychology*, **75**, 197–220.

Hirnstein, M., Hausmann, M. & Gunturkun, O. 2008. The evolutionary origins of functional cerebral asymmetries in humans: does lateralization enhance parallel processing? *Behavioural Brain Research*, **187**, 297–303.

Hopkins, W. D. 1999. Heritability of hand preference in chimpanzees (*Pan troglodytes*): evidence from a partial interspecies cross-fostering study. *Journal of Comparative Psychology*, **113**, 307–313.

Hopkins, W. D., Dahl, J. F. & Pilcher, D. 2000. Birth order and left-handedness revisited: some recent findings in chimpanzees (*Pan troglodytes*) and their implications for developmental and evolutionary models of human handedness. *Neuropsychologia*, **38**, 1626–1633.

Hopkins, W. D., Dahl, J. F. & Pilcher, D. 2001. Genetic influence on the expression of hand preferences in chimpanzees (*Pan troglodytes*): evidence in support of the right-shift theory and developmental instability. *Psychological Science*, **12**, 299–303.

Hori, M. 1993. Frequency-dependent natural-selection in the handedness of scale-eating cichlid fish. *Science*, **260**, 216–219.

Hori, M., Ochi, H. & Kohda, M. 2007. Inheritance pattern of lateral dimorphism in two cichlids (a scale eater, *Perissodus microlepis*, and an herbivore, *Neolamprologus moorii*) in Lake Tanganyika. *Zoological Science*, **24**, 486–492.

Iwasaki, S. 2000. Age and generation trends in handedness: an eastern perspective. In M. K. Mandal, M. B. Bulman-Fleming & G. Tiwari, eds. *Side bias: a neuropsychological perspective*, pp. 83–100. Dordrecht, The Netherlands: Kluwer.

Iwasaki, S., Kaiho, T. & Iseki, K. 1995. Handedness trends across age-groups in a Japanese sample of 2316. *Perceptual Motor Skills*, **80**, 979–994.

James, W. H. 1983. Twinning, handedness and embryology. *Perceptual Motor Skills*, **56**, 721–722.

Jansen, A., Lohmann, H., Scharfe, S., Sehlmeyer, C., Deppe, M. & Knecht, S. 2007. The association between scalp hair-whorl direction, handedness and hemispheric language dominance: is there a common genetic basis of lateralization? *Neuroimage*, **35**, 853–861.

Johnson, M. H., Bolhuis, J. J. & Horn, G. 1985. Interaction between acquired preferences and developing predispositions during imprinting. *Animal Behaviour*, **33**, 1000–1006.

Jones, G. V. & Martin, M. 2000. A note on Corballis (1997) and the genetics and evolution of handedness: developing a unified distributional model from the sex-chromosomes gene hypothesis. *Psychological Review*, **107**, 213–218.

Jordan, H. E. 1911. The inheritance of left-handedness. *Journal of Heredity* **2(1)**, 19–29.

Kamping, A. & Van Delden, W. 1999. A long-term study on interactions between the Adh and aGpdh allozyme polymorphisms and the chromosomal inversion In (2L)t in a seminatural population of *D. melanogaster*. *Journal of Evolutionary Biology*, **12**, 809–821.

Klar, A. J. S. 1996. A single locus, RGHT, specifies preference for hand utilization in humans. *Cold Spring Harbor Symposia on Quantitative Biology*, **61**, 59–65.

Konishi, Y., Mikawa, H. & Suzuki, J. 1986. Asymmetrical head-turning of preterm infants: some effects on lateral postural and functional lateralities. *Developmental Medicine and Child Neurology*, **28**, 450–457.

Konishi, Y., Kuriyama, M., Mikawa, H. & Suzuki, J. 1987. Effect of body position on later postural and functional lateralities of preterm infants. *Developmental Medicine and Child Neurology*, **29**, 751–757.

Kuo, Z.-Y. & Shen, T. C. 1937. Ontogeny of embryonic behavior in Aves. XI. Respiration in the chick embryo. *Journal of Comparative Psychology*, **24**, 49–58.

Laland, K. N., Kumm, J., Vanhorn, J. D. & Feldman, M. W. 1995. A gene-culture model of human handedness. *Behavior Genetics*, **25**, 433–445.

Lansky, L. M., Feinstein, H. & Peterson, J. M. 1988. Demography of handedness in 2 samples of randomly selected adults (N=2083). *Neuropsychologia*, **26**, 465–477.

Laval, S. H., Dann J.C., Butler R.J., Loftus J., Rue J., Leask S.J., Bass N., Comazzi M., Vita A., Nanko S., Shaw S., Peterson P., Shields G., Smith A.B., Stewart J., DeLisi L.E., Crow T.J. 1998. Evidence for linkage to psychosis and cerebral asymmetry (relative hand skill) on the X chromosome. *American Journal of Medical Genetics B*, **81**, 420–427.

Leiber, L. & Axelrod, S. 1981. Intra-familial learning is only a minor factor in manifest handedness. *Neuropsychologia*, **19**, 273–288.

Levin, M. 1999. Twinning and embryonic left-right asymmetry. Laterality, 4, 197–208.

Levy, J. 1974. Psychobiological implications of bilateral asymmetry. In J. Dimond & J. G. Beaumont, eds. *Hemispheric function in the human brain*, pp. 121–183. London, UK: Paul Elek.

Levy, J. & Nagylaki, T. 1972. Model for genetics of handedness. *Genetics*, **72**, 117–128.

Llaurens, V., Raymond, M. & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 881-894

Lonsdorf, E. V. & Hopkins, W. D. 2005. Wild chimpanzees show population-level handedness for tool use. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 12 634–12 638.

Manns, M. & Gunturkun, O. 1999. Monocular deprivation alters the direction of functional and morphological asymmetries in the pigeon's (*Columba livia*) visual system. *Behavioral Neuroscience*, **113(6)**, 1257–1266.

McKeever, W. F. 2000. A new family handedness sample with findings consistent with X-linked transmission. *British Journal of Psychology*, **91**, 21–39.

McManus, I. C. 1985a. Handedness, language dominance and aphasia: a genetic model. *Psychological Medicine*, **8**, 1–40.

McManus, I. C. 1985b. Right-hand and left-hand skill: failure of the right shift model. *British Journal of Psychology*, **76**, 1–16.

McManus, I. C. 1991. The inheritance of left-handedness. *Ciba Foundation symposium*, **162**, 251–281.

McManus, I. C. 1992. Are paw preference differences in hi and lo mice the result of specific genes or of heterosis and fluctuating asymmetry? *Behavior Genetics*, **22**, 435–451.

McManus, I. C. 1999. Handedness, cerebral lateralization, and the evolution of language. In M. C. Corballis & S. E. G. Lea, eds. *The descent of mind: psychological perspectives on hominid evolution*, pp. 194–217. Oxford, UK: University Press.

McManus, I. C. 2002. Right hand, left hand. London, UK: Widenfeld and Nicolson.

McManus, I. C. & Bryden, M. P. 1992. The genetics of handedness, cerebral dominance and lateralization. In I. Rapin & S. J. Segalowitz, eds. *Handbook of neuropsychology*, pp. 115–144. Amsterdam, The Netherlands: Elsevier.

Medland, S. E., Wright, M. J., Geffen, G. M., Hay, D. A., Levy, F., Martin, N. G. & Duffy, D. L. 2003. Special twin environments, genetic influences and their effects on the handedness of twins and their siblings. *Twin Research*, **6**, 119–130.

Michel, G. F. 1981. Right-handedness: a consequence of infant supine head-orientation preference? *Science*, **212**, 685–687.

Michel, G. F. & Goodwin, R. 1979. Intrauterine birth position predicts newborn supine head position preferences. *Infant Behavior and Development*, **2**, 29–38.

Michel, G. F., Tyler, A. N., Ferre, C. & Sheu, C.-F. 2006. The manifestation of infant hand-use preferences when reaching for object during the seven-to thirteen-month age period. *Developmental Psychobiology*, **48**, 436–443.

Morgan, M. J. & Corballis, M. C. 1978. Biological basis of human laterality. 2. Mechanisms of inheritance. *Behavioral and Brain Sciences*, **1**, 270–277.

Newman, H. H. 1928. Studies of human twins. II. asymmetry reversal of mirror imaging in identical twins. *Biological Bulletin*, **55**, 298–315.

Nottebohm, F. 1970. Lateralization of bird song. *Science*, **170**, 1333–1335.

Nottebohm, F. 1971. Neural lateralization of vocal control in a passerine bird. I. Song. *Journal of Experimental Zoology*, **177**, 229–261.

Nottelmann, F., Wohlslager, A. & Gunturkun, O. 2002. Unihemispheric memory in pigeons: knowledge, the left hemisphere is reluctant to share. *Behavioural Brain Research*, **133**, 309–315.

Oppenheim, R. W. 1973. Prehatching and hatching behavior: comparative and physiological consideration. In G. Gottlieb, ed. *Behavioral embryology*, pp. 161–244. New York, NY: Academic Press.

Orlebeke, J. F., Knol, D. L., Koopmans, J. R., Boomsma, D. I. & Bleker, O. P. 1996. Left-handedness in twins: genes or environment? *Cortex*, **32**, 479–490.

Pfannkuche, K. A., Bouma, A. & Groothuis, T. G. G. 2009. Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**. 929-942

Porac, C. & Coren, S. 1981. Lateral preferences and human behavior. New York, NY: Springer.

Porac, C., Coren, S. & Searleman, A. 1986. Environmental-factors in hand preference formation: evidence from attempts to switch the preferred hand. *Behavior Genetics*, **16**, 251–261.

Previc, F. H. 1991. A general theory concerning the prenatal origins of cerebral lateralization in humans. *Psychological Review*, **98**, 299–334.

Provins, K. A. 1997. Handedness and speech: a critical reappraisal of the role of genetic and environmental factors in the cerebral lateralization of function. *Psychological Review*, **104**, 554–571.

Rice, T., Plomin, R. & DeFries, J. C. 1984. Development of hand preference in the Colorado adoption project. *Perceptual Motor Skills*, **58**, 683–689.

Riedstra, B. 2003. Development and social nature of feather pecking. PhD thesis, University of Groningen.

Rife, D. C. 1940. Handedness, with special reference to twins. *Genetics*, **25**, 178–186.

Rogers, L. J. 1982. Light experience and asymmetry of brain function in chickens. Nature, 297, 223–225.

Rogers, L. J. 1990. Light input and the reversal of functional lateralization in the chicken brain. *Behavioural Brain Research*, **38**, 211–221.

Rogers, L. J. 1995. The development of brain and behaviour in the chicken. Wallingford, UK: CAB International.

Rogers, L. 1996. Behavioral, structural and neurochemical asymmetries in the avian brain: a model system for studying visual development and processing. *Neuroscience and Biobehavioral Reviews*, **20**, 487–503.

Rogers, L. J. & Workman, L. 1993. Footedness in birds. *Animal Behaviour*, **45**, 409–411.

Ronnqvist, L. & Hopkins, B. 2000 Motor asymmetries in the human newborn are state dependent, but independent of position in space. *Experimental Brain Research*, **134**, 378–384.

Ronnqvist, L., Hopkins, B., Van Emmerik, R. & de Groot, L. 1998. Lateral biases in head turning and Moro response in the human newborn: are they both vestibular in origin? *Developmental Psychobiology*, **33(4)** , 339–349.

Shafey, T. M. 2004. Effect of lighted incubation on embryonic growth and hatchability performance of two strains of layer breeder eggs. *British Poultry Science*, **45**, 223–229.

Shafey, T. M. & Al-Mohsen, T. H. 2002. Embryonic growth, hatching time and hatchability performance of meat breeder eggs incubated under continuous green light. Asian–Austr. *Journal of Animal Science*, **15**, 1702–1707.

Sicotte, N. L., Woods, R. P. & Mazziotta, J. C. 1999. Handedness in twins: a meta-analysis. *Laterality*, **4**, 265–286.

Smart, J. L., Jeffery, C. & Richards, B. 1980. A retrospective study of the relationship between birth history and handedness at 6 years. *Early Human Development*, **4**, 79–88.

Sommer,I.E.C., Ramsey, N. F., Bouma, A. &Kahn, R. S. 1999. Cerebral mirror-imaging in a monozygotic twin. *Lancet*, **354**, 1445–1446.

Soper, H. V. & Satz, P. 1984. Pathological left-handedness and ambiguous handedness: a new explanatory model. *Neuropsychologia*, **22**,511–515.

Stocks, P. 1933. A biometric investigation of twins and their brothers and sisters. *Annals of Eugenics*, **5**, 1–55.

Subirana, A. 1964. The relationship between handedness and cerebral dominance. *Journal of Neurology*, **4**, 215–234.

Templeton, J. J. & Gonzalez, D. P. 2004. Reverse lateralization of visual discriminative abilities in the European starling. *Animal Behaviour*, **76**, 783–788.

ten Cate, C. 1991. Population lateralization in zebra finch courtship: a re-assessment. *Animal Behaviour*, **41**, 900–901.

ten Cate, C., Baauw, A., Ballintijn, M. R., Majoor, B. & Van der Horst, I. 1990. Lateralization of orientation in sexually active zebra finches: eye use asymmetry or locomotor bias? *Animal Behaviour*, **39**, 992–995.

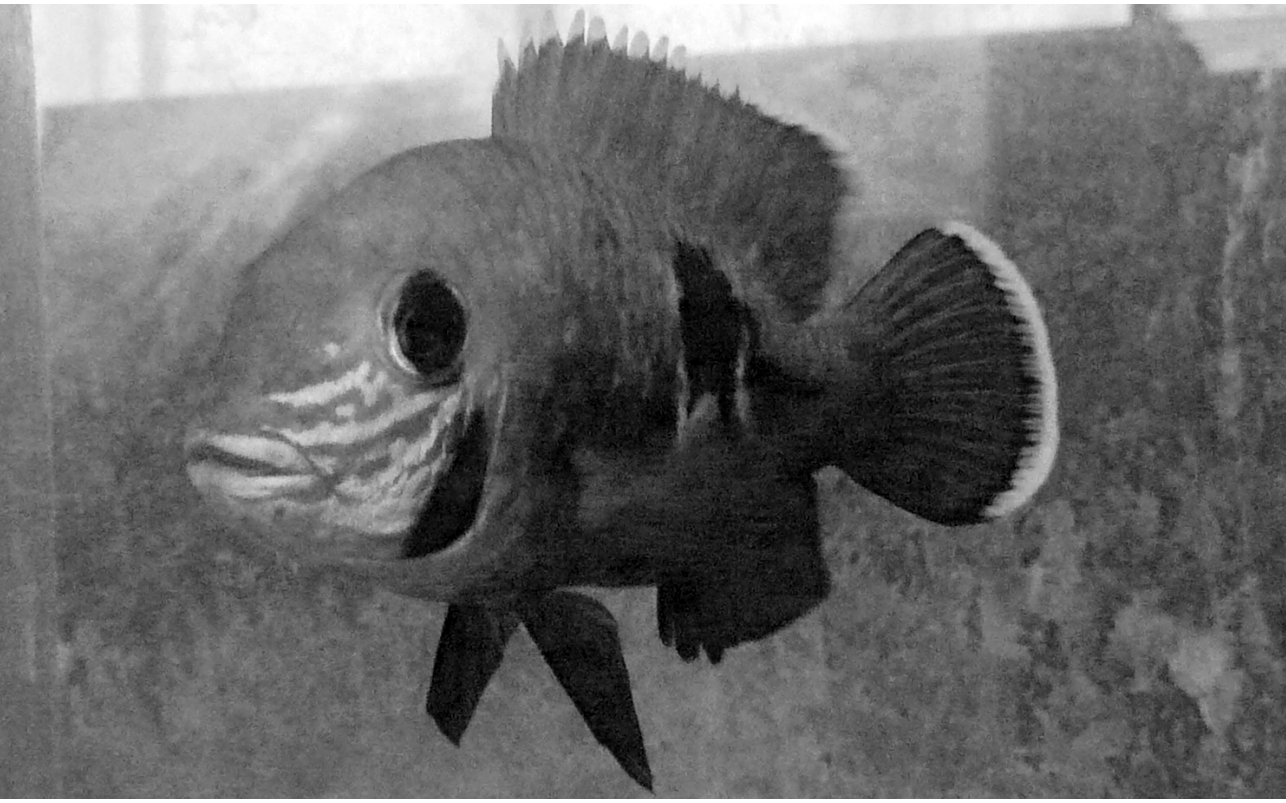
Turkewitz, G., Moreau, T. & Birch, H. G. 1966. Head position and receptor organization in the human neonate. *Journal of Experimental Child Psychology*, **4**, 169–177.

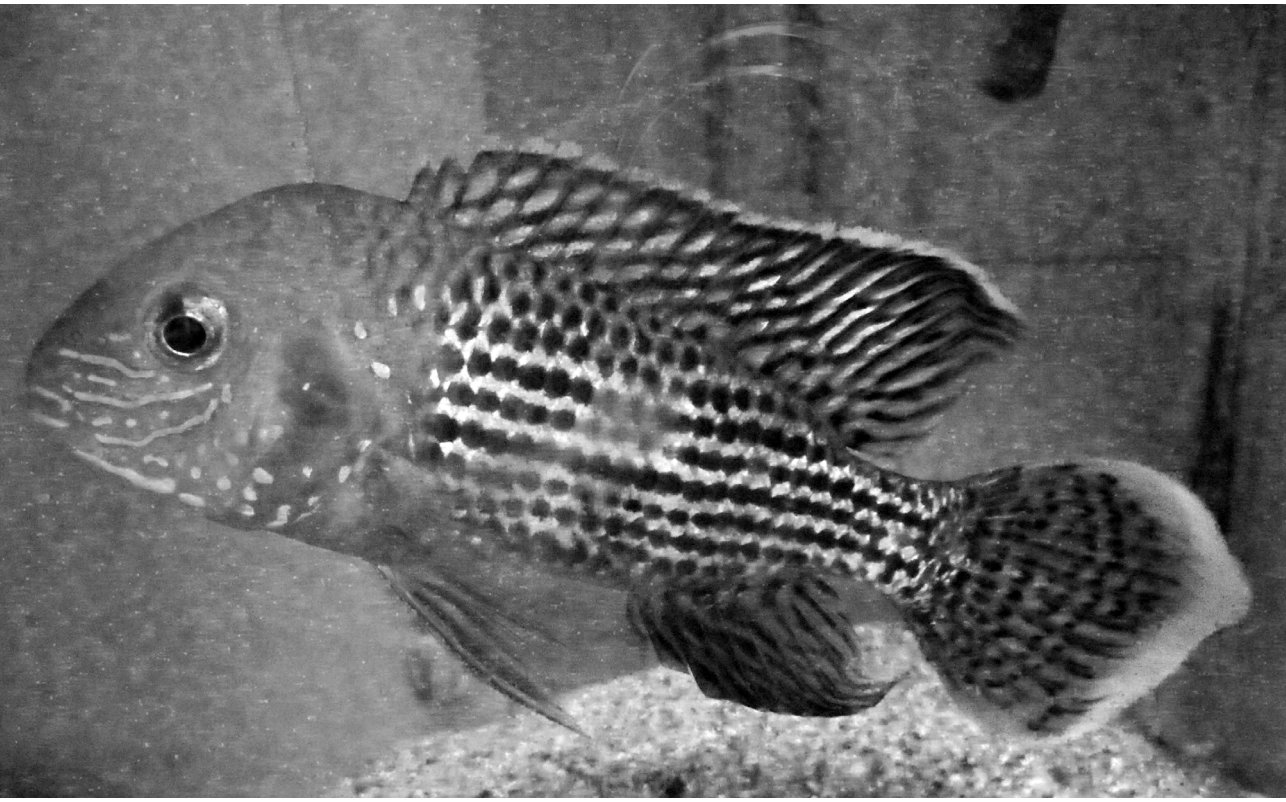
Vallortigara, G. & Rogers, L. J. 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences*, **28**, 575–633.

Vallortigara, G., Regolin, L. & Pagni, P. 1999. Detour behaviour, imprinting and visual lateralization in the domestic chick. *Cognitive Brain Research*, **7**, 307–320.

Van Agtmael, T., Forrest, S. M., Del-Favero, J., Van Broeckhoven, C. & Williamson, R. 2003. Parametric and nonparametric genome scan analyses for human handedness. *European Journal of Human Genetics* **11**, 779–783.

Yeo, R. A. & Gangestad, S. W. 1993. Developmental origins of variation in human hand preference. *Genetica,* **89**, 281–296.





3

Sex-specific effects of maternal testosterone on lateralization in cichlid fish

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Abstract

Lateralization of cerebral functions is a fundamental aspect of the organization of brain and behaviour in vertebrates. Sex differences in human lateralization have inspired researchers to postulate several hypotheses concerning the effect of prenatal testosterone on lateralization, but few experimental studies have examined these hypotheses. We investigated whether prenatal testosterone affects strength or direction of lateralization in a cichlid fish, *Aequidens rivulatus*. Eggs were given a control or testosterone treatment immediately after spawning, mimicking elevated maternal androgen concentrations towards the high end of the natural range. After 7 months the fish were tested in two rotational preference tests. As expected from earlier studies, control fish showed (nonsignificant) right-eye preference while viewing a predator and (significant) left-eye preference while viewing their mirror image, but no clear sex differences were apparent. A sex-specific effect of our treatment was found in the first test. Only females exposed to elevated prenatal levels of testosterone significantly shifted in direction of lateralization. In the second test no effect of the treatment was found. Our results suggest that mothers have a stronger influence on the lateralization pattern of their daughters than on their sons, but do not support any of the current hypotheses about prenatal testosterone and development of lateralization.

Introduction

Lateralization, the asymmetric specialization of functions in the brain, is apparent in most, if not all, vertebrate animals (Vallortigara & Bisazza 2002; MacNeilage *et al.* 2009; Vallortigara *et al.* 2011). In humans, sex differences in lateralization (for a meta-analysis see Papadatou-Pastou *et al.* 2009) has inspired researchers to hypothesize that prenatal testosterone (pT) is involved in the development of lateralization (reviewed in Pfannkuche *et al.* 2009). One well-cited hypothesis was put forward by Geschwind & Galaburda (1985) and proposed that pT slows neuronal growth in the left hemisphere and consequently leads to compensatory growth in the right hemisphere. Increased levels of pT would thus lead to increased dominance of the right hemisphere. This hypothesis has been extensively criticized in the literature (e.g. Bryden *et al.* 1994; Berenbaum & Denburg 1995). Another hypothesis, the sexual differentiation hypothesis, proposes that lateralization is related to the process of sexual differentiation and that early exposure to testosterone causes masculinization of lateralization (Hines & Shipley 1984). Finally, the callosal hypothesis states that pT increases axonal pruning in the corpus callosum of males which leads to a decrease in communication between the hemispheres and therefore increases the strength of lateralization (Witelson & Nowakowski 1991). This hypothesis only applies to mammals, as nonmammals have no anatomic structure homologous to the corpus callosum.

Although these hypotheses were inspired by sex differences in human lateralization, testing them on humans is limited by ethical concerns and human research has therefore focused on an observational approach. Unfortunately this approach, using either nonrandom clinical samples of individuals with abnormal prenatal exposure to gonadal hormones or indirect markers for pT, has lead to ambiguous results. Some studies, using clinical samples, found that girls prenatally exposed to higher levels of testosterone show enhanced left-hemisphere dominance of language (Hines & Shipley 1984), whereas others found no effect on language lateralization (Mathews *et al.* 2004; Smith & Hines 2000). Similar studies found that high pT leads to reduced right-hand preference (Schachter 1994; Mathews *et al.* 2004), others to increased left-handedness (Nass 1987; Scheirs & Vingerhoets 1995; Kelso *et al.* 2000; Smith & Hines 2000) and one study found no effect on handedness (Helleday *et al.* 1994). Furthermore some studies, using indirect markers for pT such as 2D:4D digit ratio, adult testosterone levels or sex of the co-twin, found a positive correlation between high levels of pT and right-handedness (Moffat & Hampson 1996; Gadea 2003), whereas others found a negative (Tan 1991) or no correlation (Beaton *et al.* 2011). Only three studies measured pT directly in amniotic fluid and correlated this with lateralization patterns of the resulting children. Again inconsistent results were found: Grimshaw *et al.* (1995) found that higher pT exposure was related to increased left-hemisphere specialization of speech in girls and not in boys whereas Lust *et al.* (2010) found a stronger effect in boys, although this did not result in a significant sex effect. Furthermore, the first study found that higher pT was associated with increased right-handedness in girls, but not in boys (Grimshaw *et al.* 1995), whereas another study found no such correlation between pT and handedness (Lust *et al.* 2011). Few studies have taken an experimental approach in investigating the

effects of pT exposure on lateralization in nonhuman animals and, in accordance with the observational studies in humans mentioned above, these studies often found sex-specific effects. Testosterone treatment administered on day 16 of incubation in chickens, *Gallus gallus domesticus*, reversed the pattern of asymmetry in the brain of males and decreased lateralization in females (Schwarz & Rogers 1992). Prenatal testosterone treatment also moderately shifted the population-level lateralization of tail posture in female Wistar rats, *Rattus norvegicus*, whereas in males there was no effect (Rosen *et al.* 1983). These studies highlight the importance of studying lateralization in general, and the effect of testosterone on lateralization in particular, with respect to the sex of the animal. A meta-analysis showed that the data available thus far, on both human and nonhuman animals, do not support any of the three hypotheses mentioned above, possibly because of the small number of experimental studies investigating the effects of pT on lateralization (Pfannkuche *et al.* 2009).

To investigate experimentally the effect of pT on lateralization and its sex specificity, we used the cichlid fish species *Aequidens rivulatus* as model organism. Cichlid fish are oviparous, facilitating easy manipulation of pT levels in the eggs outside the mother's body. The pT can originate from both the embryo itself and from its mother. Congruent with avian species (reviewed in von Engelhardt & Groothuis 2011), egg yolk of fish contains substantial levels of maternal steroid hormones, including androgens (Schreck *et al.* 1991; McCormick 1998; Eriksen *et al.* 2006) that can affect offspring development in several fish species (McCormick 1999; Gagliano & McCormick 2009; Sloman 2010). Since maternal hormone production is under the influence of the environment, a mother can communicate the prevailing conditions to her offspring by means of depositing different amounts of gonadal hormones into her eggs, influencing physiology, morphology and behaviour, often in a sex-specific manner (birds: reviewed in Groothuis *et al.* 2005; Gil 2008; von Engelhardt & Groothuis 2011). In the current study we tested whether elevation of testosterone concentrations in the eggs immediately after spawning, mimicking elevated deposition of maternal androgens, affects lateralization of cichlid fish species sex specifically. Determination of lateralization is very easy in teleost fish, as they have laterally placed eyes, small overlapping visual fields, visual pathways that project almost entirely to the contralateral hemisphere and low communication between the two hemispheres (Vanegas & Ito, 1983). Fish are known to use the left eye to view their mirror-image (Sovrano et al 1999, 2001; De Santi *et al.* 2001; Sovrano & Andrew 2006; but see Reddon & Balshine 2010) and the right eye to view a predator (Bisazza & Vallortigara 1997; Bisazza *et al.* 1998, 1999; Facchin *et al.* 1999; Brown *et al.* 2004). We therefore used these two stimuli to investigate the effect of our treatment on the strength and direction of lateralization.

The hypothesis postulated by Geschwind & Galaburda (1985), proposing that pT causes an increase in dominance of the right hemisphere, predicts that fish that were prenatally exposed to higher levels of testosterone will show an even stronger bias, compared to control fish, to view their mirror image with their left eye (thus increasing the strength of lateralization) as the left-eye system is controlled by the right hemisphere. Furthermore, these testosterone-treated fish would shift direction from viewing the predator with the right eye towards the left eye, which may result in a decrease in strength of lateralization. The sexual differentiation theory predicts that in pT-treated

fish both sexes will show an increased masculinized lateralization pattern, as, like in mammals, testosterone influences masculinization during the process of sexual differentiation in fish (Devlin & Nagahama 2002). However, the literature on sex differences in lateralization of fish is scarce and ambiguous. Reddon & Hurd (2009) found no sex difference in the direction of lateralization in a detour test for viewing objects that had positive or negative emotional valence, but both Bisazza *et al.* (1998) and Sovrano *et al.* (1999) found a sex difference with females showing a population bias to view same-sex individuals and their mirror image with the left eye, whereas males did not show population-level lateralization, although this could be accounted for by a lack of social behaviour in males. The opposite was found by Brown *et al.* (2007) who found a sex difference with males showing a population bias to look at a conspecific with the right eye whereas this was not present in females. Although these results are ambiguous they show that a sex difference in lateralization when viewing a conspecific is often found in fish. The sexual differentiation theory predicts that pT-treated females will show a more masculine pattern of lateralization than control females when viewing their mirror image. The lateralization pattern of the pT-treated females will thus resemble more the lateralization pattern of control males. As no sex effect in lateralization has been found when viewing a predator (e.g. Bisazza *et al.* 1998) no effect of treatment is expected in this task. As fish have no anatomic structure homologous to the corpus callosum, the callosal hypothesis will not be considered, as it cannot be tested in this species.

Methods

Subjects, housing and experimental design

Aequidens rivulatus, a sexually dimorphic, substrate-breeding cichlid fish originating from Ecuador and Peru (Stawikowski & Werner, 1998) were bred in 200 litre tanks as the second generation of wild-caught fish at our laboratory (for details see Schaafsma & Groothuis 2011/chapter 4, this thesis). These fish, like most cichlids, show elaborate schooling behaviour before reaching sexual maturity and elaborate display behaviour during social interactions. *Astronotus ocellatus*, also a South American cichlid species, was obtained from a local pet store (four individuals) and used as a predator stimulus in the rotational preference task (see below). All adult fish were held in pairs and fed dry fish flakes (TetraMin Tropical Fish Flakes) daily and additionally received frozen shrimps weekly. All tanks were connected to large biological water filtration systems in which water temperature was kept at 25±2 °C. The light:dark schedule was 12:12h.

Eight pairs of *A. rivulatus* were used to obtain eight clutches. Clutches are laid just before lights-off and spawning was checked shortly before that time. Eggs were laid on shale covered with thin polypropylene sheets. Immediately after the discovery of a clutch the shale containing the eggs was gently removed from the tank to minimize parental disturbance and immediately replaced by shale covered with clean polypropylene sheets. We then collected the eggs and started the treatment. Of these clutches half of the eggs received testosterone treatment and half a control treatment (see below). After treatment of the eggs, they were left to hatch in compartments measuring ca. 20 x 30 cm and 20 cm high. Fry and larvae, housed in groups split according to clutch and

treatment, were fed daily with brine shrimp. We tested the fish at 7 months posthatching (standard length mean ± SD = 3.06 ± 0.061 cm), before they became reproductively active. We used 92 fish, originating from four clutches, produced by four different females and males; only four clutches were used because these were the only ones that contained sufficient fish for both control and testosterone-treated groups. The fish underwent both a rotational preference test with a live predator as stimulus (RPT-P) and a rotational preference test in which the fish could view their own mirror image (RPT-M; see below). Half of the fish first performed the RPT-P and the other half first performed the RPT-M. After the first test the fish were gently dip-netted out of the test apparatus and placed into a small opaque PVC tube (diameter 5 cm) for acclimatization in the other test apparatus for 5 min. After the last test the fish were killed for sex determination (see below).

Treatment of eggs

The freshly laid clutches were gently taken off from the polypropylene sheet using a scalpel and divided in two, and placed in well-aerated 200 ml cups containing home tank water. One half of the clutch received the testosterone treatment, which was based on a pilot study on 11 clutches. In this pilot study we split every clutch into four and used four different dosages (0, 2, 20 or 200 μg of methyl-testosterone (MT, Sigma) dissolved in 0.1 ml of ethanol per litre of tank water) within a clutch to establish which concentration elevates the mean levels of testosterone to two times the standard deviation of control clutches.

Based on the results of this pilot study (0 μg MT: mean ± standard deviation = 2.65 ± 2.51 μg; 2 μg MT: mean ± standard deviation = 5.46 ± 3.92 μg; 20 μg MT: mean ± standard deviation = 72.07 ± 56.86 μg; 200 μg MT: mean ± standard deviation = 758.27 ± 561.10 μg) we used 3.57 μg of methyl-testosterone (Sigma) dissolved in 0.1 ml of ethanol per litre of tank water as the testosterone treatment. The other half of the clutch received a control treatment (0.1 ml of ethanol per liter of tank water). After 24 h of treatment, the eggs were collected and rinsed with clean water. Half of the testosterone-treated and control eggs were frozen for hormone analyses and the other half was left to hatch.

Rotational preference test with predator

This test was based on the test developed by Bisazza *et al.* (1997) and was mostly identical to the procedure of the rotational preference test in the study of Schaafsma & Groothuis (2011/chapter 4, this thesis). In short, it consisted of a circular tank (diameter 48 cm) of which the inside was covered by black polypropylene (Vikuprop, Vink Kunststoffen BV, Didam, The Netherlands). In the middle of the tank a smaller transparent circular tank was placed (diameter 20 cm) which contained the predator (of which four individuals were used in total; standard length mean ± standard deviation = 13.02 ± 0.62 cm) restrained from moving by means of two Plexiglas plates. The tank was filled with 15 cm of water and lit from above with a 60 W light bulb. Two of these identical devices were placed in a completely darkened room. The subject fish were allowed to acclimatize in a small opaque PVC tube (diameter 5 cm), which was positioned anterior to the predator for 5 min. Thereafter, the light was switched on, the tube was gently removed, and behaviour recorded for 15 min. The recordings were analysed using the software

program Observer 6.0 (Noldus Information Technology, Wageningen, The Netherlands). Time spent swimming in a clockwise or anticlockwise position was computed. We excluded from analysis the time fish spent turning to change their viewing position or trying to escape from the apparatus (facing the outer wall).

Rotational preference test with mirror

This test consisted of a circular tank (diameter 30 cm) with mirror foil attached to the inner wall. The tank was filled to the edge of the mirror foil with water (15 cm). The subject fish was gently dip-netted into a small opaque PVC tube (diameter 5 cm) placed in the middle of the apparatus and left to acclimatize for 5 min in total darkness. Then the light was turned on, the tube gently removed, and behaviour recorded for 15 min. The recordings were analysed in the same way as described for the RPT-P.

Sex determination

Immediately after the behavioural tests the fish were placed in a 200 ml opaque cup containing a clove oil and water mixture that had been thoroughly mixed, a technique commonly used to euthanize fish (see section Ethical note below). After euthanasia the fish were decapitated and immediately frozen at -80 °C. At the time of histology the euthanized fish were taken out of the freezer, tail and epaxial musculature were removed and transverse serial sections were cut at 12 μm, stained with haematoxylin-eosin Y and examined under a light microscope. We scored the presence of ovarian or testicular tissue. Hermaphrodite fish were not found. Sex determination revealed 22 males (12 controls) and 44 females (19 controls). In 26 fish no ovarian or testicular tissue was found.

Determination of egg hormone levels

Egg testosterone levels were quantified by radioimmunoassay after extraction. First, hormones were extracted from egg samples using a modification of a protocol used previously on salmonid eggs (Eriksen *et al.* 2006). Briefly, samples were weighed (to the nearest 0.001 g), and crushed in a glass Potter homogenizer after adding 500 μl of 100% methanol. The sample was transferred to a glass tube, and the Potter tube and rod were each rinsed twice with 500 μl of 100% methanol and then added to the glass tube. The sample was then dried under nitrogen at 50 °C, and extracted in 3 ml of 70% diethyl ether (DEE) and 30% petroleum benzine (PB), vortexed for 60 s and centrifuged (2000 rpm, 5 min, 4 °C). This process was repeated twice (addition of 3 ml 70% DEE/30% PB, 30 s and 15 s vortex, respectively). All extracts from each sample were combined and dried under nitrogen at 37 °C. Next, 2 ml of 70% methanol was added and the sample was vortexed and left for 3 days at -20°C. Then, the extracts were centrifuged for 5 min (2000 rpm, 4 °C), decanted and dried under nitrogen at 50 °C. Subsequently, extracts were dissolved in 150 μl of phosphate-buffered saline with gelatine. From this solution, a subsample of 20 μl was taken, mixed with scintillation cocktail (Ultima Gold, Perkin Elmer) and radioactivity (^{3}H, Perkin Elmer) counted on a liquid scintillation counter. Standards for each assay were prepared using dilution series from preprepared stock and ranged from 0.04 to 20 ng/ml of testosterone. Egg dilution curves ran parallel to the standards. Intra-assay CV was 3%.

Statistical analysis

Five of the 22 males (two testosterone) and five of the 44 females (all testosterone) were immobile during the RPT-P and were removed from further analyses. In the RPT-M five males (four testosterone) and five females (four testosterone) were immobile during the test and were removed from further analyses.

Following the literature (e.g. Bisazza & Vallortigara 1997), we calculated a laterality index (LI). In the RPT-P the LI was calculated using the formula: (time spent swimming anticlockwise – time spent swimming clockwise)/(time spent swimming anticlockwise + time spent swimming clockwise). In the RPT-M the LI was calculated as: (time spent swimming clockwise – time spent swimming anticlockwise)/(time spent swimming clockwise + time spent swimming anticlockwise). In this manner, the formulas calculating the LIs in the RPT-P and RPT-M both resulted in positive values when the left eye was used more, and negative values when the right eye was used more, because in the RPT-P test anticlockwise swimming indicates fixating the predator with the left eye while in the RPT-M test anticlockwise swimming indicates viewing itself in the mirror with the right eye.

Also, the absolute value of the laterality index was calculated to investigate the strength of lateralization, independent of the direction.

After we applied an arcsine square-root transformation the LIs followed a normal distribution. The log (x x 100 + 1) transformations of the absolute values of the LI were not completely successful in normalizing the data, but the residuals of the models (in contrast to the residuals of the models when the untransformed variable was used) followed a normal distribution. Variances of the dependent variables were not significantly different across groups in all models. The LI and |LI| were analysed using hierarchical models in the software program MLwiN 2.02 (Rasbash *et al*. 2005). We created two levels, the highest being clutch ID and the lowest fish ID. Treatment and sex were the categorical predictive factors. The models were estimated using restricted iterative generalized least squares. Significance of the fixed factors was tested using the Wald statistic, which follows a chi-square distribution.

To test whether groups showed a population bias of lateralization, we conducted one-sample t tests. To investigate whether our hormone treatment was effective we used a Wilcoxon signed-ranks test, as hormone concentrations concerned paired, non-normally distributed data. These statistical analyses were performed in the software program SPSS 16.0.2 (SPSS Inc., Chicago, IL, U.S.A.). All tests were two tailed and significance level was set at $\alpha<0.05$.

Ethical note

Hatching success and survival of the larvae over the first few days after hatching was low in four out of eight clutches, irrespective of treatment. As clutches were not attended by their parents, which would normally fan and clean the eggs, this lack of parental care might have been the primary cause. We had no indications that the welfare of fish that were exposed to pT levels elevated within their physiological range was impaired. No adverse effects of testosterone were seen on survival or growth. Fish were

killed by an overdose of clove oil (1 g/litre). We used clove oil in more than five times the anaesthetic solution (maximum 100 mg/litre for induction in fish, Neiffer & Stamper 2009). Clove oil has been shown to be an appropriate anaesthetic in fish, inducing anaesthesia quicker than MS-222 (Perdikaris *et al*. 2010) and is a recommended method for euthanasia in fish (ANZCCART 2001). Opercular movement ceased within 1 min and fish were left in the solution additionally for at least 10 min. After euthanasia the fish were decapitated and immediately frozen at -80 °C.

The predators, being hand tame and very calm in our tanks, were restrained during the behavioural trials by means of two Plexiglas plates to prevent any lateralized interactions between the predator and the subject fish. These plates were loosely placed in such a manner that the fish could still move but could not turn. The predators showed tonic immobility when first placed in this position, but after approximately 2 min showed a normal posture and no changes to a darker colour (a sign of stress in many cichlid fish) were noted. Individual *A. ocellatus* were used for a maximum of 1 h and reused after a 47 h period of recovery in the home tank. All experimental protocols were approved by the ethical committee for animal research of the University of Groningen.

Results

Treatment of eggs

The testosterone treatment significantly affected the concentration of testosterone in the eggs after 24 hours (control: median = 1.15 pg/mg: testosterone: median = 3.13 pg/mg; Wilcoxon signed-ranks test: Z = -1.960, N = 8, $p < 0.050$). The testosterone-treated eggs showed an increase in testosterone 2.76 times the standard deviation of the control eggs (standard deviation = 0.717).

LI during rotational test with predator

No sex effect in LI was apparent in the control fish ($\chi^2_1 = 0.149$, $p = 0.699$). Although the control fish viewed the predator as expected mainly with their right eye (Figure 1a), this population bias was not significant (one sample $t_{37} = 0.152$, $p = 0.880$). However, we did find a sex-specific treatment effect. Control females viewed the predator mainly with their right eye whereas testosterone-treated females looked at the predator mainly with their left eye resulting in a significant treatment effect ($\chi^2_1 = 4.764$, $p = 0.029$). Males did not react to the treatment ($\chi^2_1 = 0.01$, $p = 0.975$) and did not show a significant preference for either eye to view the predator (figure 1a). Nevertheless, there was no significant interaction effect between treatment and sex present on LI ($\chi^2_3 = 5.630$, $p = 0.131$), possibly due to low sample size. Post-hoc tests show that testosterone females showed a significant population bias to look at the predator with their left eye (one-sample $t_{19} = 2.433$, $p = 0.025$,) whereas none of the other groups showed a significant population bias (all p values > 0.477, Figure 1a).

Control females showed a non-significant trend to be stronger lateralized than control males ($\chi^2_1 = 3.148$, $p = 0.076$, Figure 1b). Treatment had no effect on the absolute laterality index in females ($\chi^2_1 = 0.117$, $p = 0.732$), or in males ($\chi^2_1 = 1.692$, $p = 0.193$), and no interaction effect between treatment and sex ($\chi^2_3 = 2.692$, $p = 0.442$; figure 1b).

LI during rotational test with mirror

No sex effect in LI was apparent in the control fish ($\chi^2_1 = 0.001$, $p = 0.975$). As expected, the control fish showed a significant population bias to view their mirror image with the left eye (one-sample $t_{40} = 2.260$, $p = 0.029$). The testosterone treated fish did not show population bias lateralization in this test (one-sample $t_{39} = 1.308$, $p = 0.198$).

No effect of treatment was detected on the laterality index in RPT-M in either sex (females: $\chi^2_3 = 0.013$, $p = 0.909$ and males: $\chi^2_1 = 1.634$, $p = 0.201$, treatment*sex: $\chi^2_3 = 1.136$, $p = 0.768$, figure 2a).

No sex effect was apparent in the absolute value of the LI in the control fish ($\chi^2_1 = 0.034$, $p = 0.853$). There was no effect of the treatment on the females ($\chi^2_1 = 0.347$, $p = 0.556$) or on males ($\chi^2_1 = 1.125$, $p = 0.289$), or an interaction effect between treatment and sex ($\chi^2_3 = 2.761$, $p = 0.430$) on the absolute value of the LI (Figure 2b).

Discussion

In this study we aimed to test the influence of maternal androgen variation in eggs on offspring lateralization by means of artificial elevation of egg testosterone concentrations within the physiological range in a cichlid fish. Results of the hormone assay showed that our treatment was successful in elevating yolk hormone levels in the upper physiological range of this species. We performed two lateralization tests of visually guided behaviour, and expected, based on the literature, that the control fish would

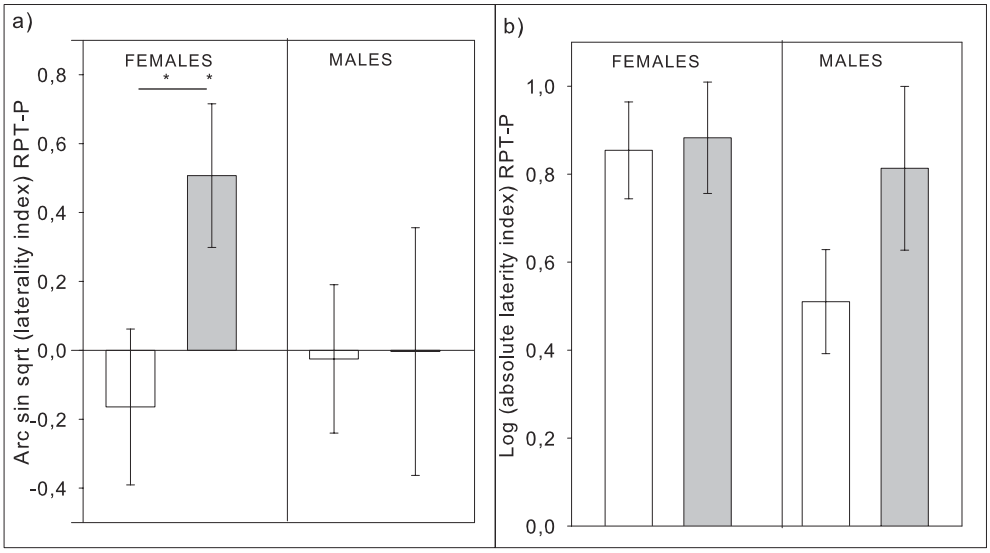

Figure 1 • (a) Laterality index (arcsine square root) and (b) absolute laterality index (log) of the rotational preference test with a predator as stimulus (RPT-P) for females (Ncontrol = 19, Ntestosterone = 20) and males (Ncontrol = 9, Ntestosterone = 8) in the control (white bars) and testosterone (grey bars) treatment groups. Positive values of the LI indicate more left-eye use. Means and standard errors of the means are shown. *: $p < 0.05$.

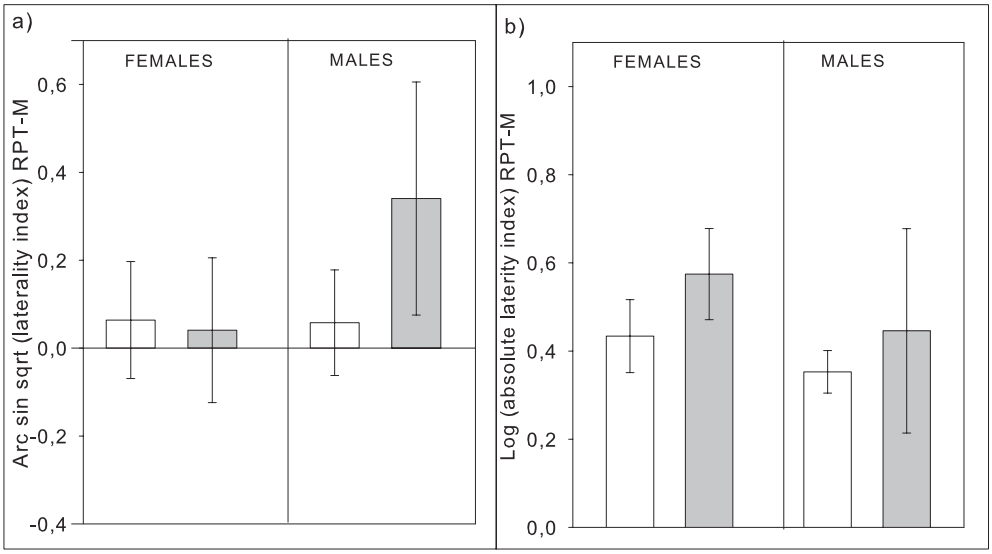

Figure 2 • (a) Laterality index (arcsine square root) and (b) absolute laterality index (log) of the rotational preference test with the fish mirror image as stimulus (RPT-M) for females (Ncontrol = 18, Ntestosterone = 21) and males ((Ncontrol = 11, Ntestosterone = 6) in the control (white bars) and testosterone (grey bars) treatment groups. Positive values of the LI indicate more left-eye use. Means and standard errors of the means are shown. *: $p < 0.05$.

view a predator mainly with the right eye (Bisazza & Vallortigara 1997, 1999; Facchin *et al.* 1999; Brown *et al.* 2004) and their mirror image with their left eye (Sovrano *et al.* 1999, 2001; de Santi *et al.* 2001; Sovrano & Andrew 2006). Although some contradictory results have also been reported concerning the latter test (right eye use for mirror viewing: Bisazza & de Santi, 2003; Reddon & Balshine 2010; Arnott *et al.* 2011), two of these studies obviously concerned lateralization of aggression and one (Reddon & Balshine 2010) used adult cichlid fish known to fight their mirror image (Riebli *et al.* 2011). Lateralization of aggression seems to be different from lateralization of viewing a conspecific, which is probably related to schooling. We analysed the results in a sex-specific way. The results of the tests show that the population bias in the control group was in the most commonly observed direction both while viewing a predator and while viewing their mirror image outside an agonistic context, but was only significant for the latter test. However, even in this mirror test the effect was subtle such that individual fish could show no bias or even the opposite bias of that at the population level.

Our results show that pT changed the direction of lateralization while viewing a predator in female, but not in male *A. rivulatus*. Control females showed a nonsignificant bias to view the predator with the right eye, whereas females that were exposed to increased levels of testosterone prenatally showed a significant population bias to view the predator with the left eye. Such a shift in the direction of lateralization in females only is in agreement with a study in Wistar rats, although lateralization in the rat study concerned motor behaviour not visually guided behaviour (Rosen *et al.* 1983). In contrast

to our finding, Schwarz & Rogers (1992) found that in chickens testosterone treatment administered on day 16 of incubation reversed the pattern of asymmetry in the brain of males whereas it decreased lateralization in females. However, these authors investigated part of the visual pathway by examining the brain anatomy of very young chicks (6 days post hatching) and not the behaviour in almost mature animals as we did.

The current study does not support the sexual differentiation hypothesis (Hines & Shipley 1984) as females did not shift in direction of lateralization towards a more masculine pattern when the fish were viewing the mirror image and the sex difference in lateralization in control fish was not significant. Our results partly support the Geschwind & Galaburda (1985) hypothesis, as we found an increase in right-hemisphere dominance in fish that were prenatally exposed to testosterone, in the predator viewing task, but this was only true for females. Moreover, this hypothesis is based on sex differences in humans, whereas we did not find such differences in control fish. Furthermore, in contrast to the prediction, no significant increase in strength of lateralization was observed when viewing the mirror image.

We did not find an effect of pT on lateralization in males, but we did in females, suggesting that the latter are more sensitive to pT exposure, or that prenatal or perinatal testosterone production by males is higher than by females and overrides the effect of maternal testosterone. In any case, our results suggest that mothers may influence the direction of lateralization of daughters but not sons. In this species, however, sons are sensitive to postnatal testosterone, which affects the direction of lateralization in males and not in females (Schaafsma & Groothuis 2011/chapter 4, this thesis), perhaps acting as a means for males to adjust their direction of lateralization.

Strength of lateralization was not affected by hormone treatment. Although in another fish species direction was shown to have a heritable component (Bisazza *et al.* 2001), in cichlid fish, strength, but not direction, of lateralization is a heritable trait (Brown *et al.* 2007). Possibly, strength of lateralization is a genetic trait, whereas direction of lateralization is under the influence of (heritable) environmental factors such as gonadal hormones.

Prenatal testosterone did not have an effect on the lateralization pattern during the rotational preference test with their mirror image as stimulus. The finding that pT affects one behavioural domain of lateralization but not another is in agreement with the human literature in which pT correlated differently with lateralization patterns of language and handedness (Lust *et al.* 2011).

Both predator–prey interactions and agonistic and synergetic interactions within species can theoretically lead to population-level lateralization (Ghirlanda & Vallortigara 2004; Ghirlanda *et al.* 2009, respectively). However, population-level lateralization in tasks that measure behaviours related to these different evolutionarily stable strategies does not have to be cross-correlated (Sovrano *et al.* 1999). Predator pressure can be highly variable in different habitats for the same species (e.g. Brown 2004; Bell & Sih 2007; Dingemanse *et al.* 2007), whereas conspecifics will always be around; thus different costs and benefits have been suggested to be associated with population-level lateralization with respect to antipredator and social behaviours. Strong population lateralization in species regarding predatory behaviour could be exploited by the predator, whereas

this disadvantage may be smaller for interspecific interactions, as it may promote coordination of schooling behaviour (Bisazza & Dadda 2005) and minimize fighting costs (Arnott *et al*. 2011). Therefore, more environmentally induced flexibility in the direction of lateralization for viewing predators than conspecifics may be adaptive.

Acknowledgements

We are grateful to Daan Buijtenhuijs for help with breeding the fish and treatment of the eggs and to Jasmijn Grootjans and Daphne Piersma for help with the behavioural tests. We thank Bonnie de Vries, Ilse Weites and the late Maarten Lasthuizen for help with the hormone assays and Sjoerd Veenstra, Roelie Wiegman-Veenstra, Saskia Helder and Christa Reitzema-Klein for excellent animal care.

References

Arnott, G., Ashton, C. & Elwood, R.W. 2011. Lateralization of lateral displays in convict cichlids. *Biology Letters*, **7**, 683-685

Beaton, A., Rudling, N., Kissling, C., Taurines, R. & Thome, J. 2011. Digit ratio (2D:4D), salivary testosterone, and handedness. *Laterality*, **16**, 136-155.

Bell, A. M. & Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, **10**, 828-834.

Berenbaum, S. A. & Denburg, S. D. 1995. Evaluating the empirical support for the Geschwind-Behan-Galaburda model of cerebral lateralization: commentary on Bryden, McManus, and Bulman-Fleming. *Brain and Cognition*, **27**, 79-83.

Bisazza, A. & Dadda, M. 2005. Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 1677-81.

Bisazza, A. & de Santi, A. 2003. Lateralization of aggression in fish. *Behavioural Brain Research*, **141**, 131-136.

Bisazza, A. & Vallortigara, G. 1997. Rotational swimming preferences in mosquitofish: evidence for brain lateralization? *Physiology & Behavior*, **62**, 1405-1407.

Bisazza, A., de Santi, A.Vallortigara, G., 1999. Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal Behaviour*, **57**, 1145-1149.

Bisazza, A., Pignatti, R. & Vallortigara, G. 1997. Detour tests reveal task-and stimulus-specific neural lateralization in mosquitofish (*Gambusia holbrooki*). *Behavioural Brain Research*, **89**, 237-242.

Bisazza, A., Sovrano, V. & Vallortigara, G. 2001. Consistency among different tasks of left-right asymmetries in lines of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia*, **39**, 1077-1085.

Bisazza, A., Facchin, L., Pignatti, R. & Vallortigara, G. 1998. Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behavioural Brain Research*, **91**, 157-164.

Brown, C. 2004. Effects of predation pressure on the cognitive ability of the poeciliid Brachyraphis episcopi. *Behavioral Ecology*, **16**, 482-487.

Brown, C., Gardner, C. & Braithwaite, V. 2004. Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proceedings of the Royal Society B: Biological Sciences*, **271**, S455-457.

Brown, C., Western, J. & Braithwaite, V. 2007. The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour*, **74**, 231-238.

Bryden, M. P., McManus, I. & Bulman-Fleming, M. B. 1994. Evaluating the empirical support for the Geschwind-Behan-Galaburda model of cerebral lateralization. *Brain and Cognition*, **26**, 103–167.

Devlin, R. H. & Nagahami, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, **208**, 191-364

Dingemanse, N. J., Wright, J., Kazem, A. J. N., Thomas, D. K., Hickling, R. & Dawnay, N. 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *The Journal of Animal Ecology*, **76**, 1128-38.

von Engelhardt, N. & Groothuis, T. 2011. Maternal hormones in avian eggs. In D. Norris & K. Lopez, eds. *Hormones and Reproduction of Vertebrates*, Volume 4, pp. 91-128. Amsterdam: Academic Press.

Eriksen, M. S., Bakken, M., Espmark, A, Braastad, B. O. & Salte, R. 2006. Prespawning stress in farmed Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. *Journal of Fish Biology*, **69**, 114-129.

Facchin, L., Bisazza, A. & Vallortigara, G. 1999. What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural Brain Research*, **103**, 229–234.

Gagliano, M. & McCormick, M. I. 2009. Hormonally mediated maternal effects shape offspring survival potential in stressful environments. *Oecologia*, **160**, 657-665.

Geschwind, N. & Galaburda, A. M. 1985. Cerebral lateralization - biological mechanisms, associations, and pathology: I-III. A hypothesis and a program for research. *Archives of Neurology*, **42**, 428–459, 521–552, 634–654.

Ghirlanda, S. & Vallortigara, G. 2004. The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 853-857.

Ghirlanda, S., Frasnelli, E. & Vallortigara, G. 2009. Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 861-866.

Gil, D. 2008. Hormones in avian eggs: physiology, ecology and behavior. *Advances in the Study of Behavior*, **38**, 337-398.

Grimshaw, G., Bryden, M. P. & Finegan, J. 1995. Relations between prenatal testosterone and cerebral lateralization in children. *Neuropsychology*, **9**, 68-79.

Groothuis, T., Müller, W., von Engelhardt, N., Carere, C. & Eising, C. 2005. Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews*, **29**, 329-352.

Helleday, J., Siwers, B. & Ritzen, E. 1994. Normal lateralization for handedness and ear advantage in a verbal dichotic-listening task in women with congenital adrenal-hyperplasia (CAH). *Neuropsychologia*, **32**, 875-880.

Hines, M. & Shipley, C. 1984. Prenatal exposure to diethylstilbestrol (DES) and the development of sexually dimorphic cognitive abilities and cerebral lateralization. *Developmental Psychology*, **20**, 81-94.

Kelso, W. M., Nicholls, M. E., Warne, G. L. & Zacharin, M. 2000. Cerebral lateralization and cognitive functioning in patients with congenital adrenal hyperplasia. *Neuropsychology*, **14**, 370-378.

Lust, J., Geuze, R. H., Van de Beek, C., Cohen-kettenis, P. T., Groothuis, T. & Bouma, A. 2010. Sex specific effect of prenatal testosterone on language lateralization in children. *Neuropsychologia*, **48**, 536-540.

Lust, J., Geuze, R., Van de Beek, C., Cohen-Kettenis, P., Bouma, A. & Groothuis, T. 2011. Differential effects of prenatal testosterone on lateralization of handedness and language. *Neuropsychology*, **25**, 581-589.

McCormick, M. I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology*, **79**, 1873-1883.

McCormick, M. I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia*, **118**, 412-422.

MacNeilage, P.F., Rogers, L.J. & Vallortigara, G. 2009. Origins of the left & right brain. *Scientific American*, **301**, 60-67.

Mathews, G., Fane, B. A., Pasterski, V. L., Conway, G. S., Brook, C. & Hines, M. 2004. Androgenic influences on neural asymmetry: handedness and language lateralization in individuals with congenital adrenal hyperplasia. *Psychoneuroendocrinology*, **29**, 810-822.

Moffat, S.D. & Hampson, E. 1996. Salivary testosterone levels in left- and right-handed adults. *Neuropsychologia*, **34**, 225-233.

Nass, R., Baker, S. & Speiser, P. 1987. Hormones and handedness: left-hand bias in female congenital adrenal-hyperplasia patients. *Neurology*, **37**, 711-715.

Neiffer, D.L. & Stamper, M.A. 2009. Fish sedation, analgesia, anesthesia, and euthanasia: considerations, methods, and types of drugs. *Institute of Laboratory Animal Resources Journal*, **50**, 343-360.

Perdikaris, C., Nathanailides, C., Gouva, E., Gabriel, U.U., Bitchava, K., Athanasopoulou, F., Paschou, A. & Paschos, I. 2010. Size-relative effectiveness of clove oil as an anaesthetic for rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) and goldfish (*Carassius auratus* Linnaeus, 1758). *Acta Veterinaria Brno*, **79**, 481–490.

Rasbash, J., Charlton, C., Browne, W. J., Healy, M. & Cameron, B. 2005. MLwiN. Version 2.02, Multilevel Models Project. London: Institute of Education, University of London.

Reddon, A. R. & Hurd, P. L. 2009. Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural Processes*, **82**, 25-29.

Reddon, A.R. & Balshine, S. 2010. Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes*, **85**, 68-71.

Reilly, J.S. (ed.) . 2001. Euthanasia of Animals Used for Scientific Purposes. ANZCCART. http:// www.adelaide.edu.au/ANZCCART/publications/Euthanasia.pdf

Riebli, T., Avgan, B., Bottini, A.M., Duc, C., Taborsky, M. & Heg, D. 2011. Behavioural type affects dominance and growth in staged encounters of cooperatively breeding cichlids. *Animal Behaviour*, **81**, 313-323.

Rosen, G. D., Berrebi, A. S., Yutzey, D. A. & Denenberg, V. H. 1983. Prenatal testosterone causes shift of asymmetry in neonatal tail posture of the rat. *Developmental Brain Research*, **9**, 99–101.

de Santi, A., Sovrano, V., Bisazza, A. & Vallortigara, G. 2001. Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Animal Behaviour*, **61**, 305-310.

Schaafsma, S. M. & Groothuis, T. G. G. 2011. Sex-specific effects of postnatal testosterone on lateralization in cichlid fish. *Animal Behaviour*, **81**, 283-288.

Schachter, S. 1994. Handedness in intrauterine exposure to Diethylstilbestrol. *Neuropsychologia*, **32**, 619-623.

Scheirs, J. & Vingerhoets, A. 1995. Handedness and other laterality indexes in women prenatally exposed to DES. *Journal of Clinical and Experimental Neuropsychology*, **17**, 725-730.

Schreck, C., Fitzpatrick, M., Feist, G. & Yeoh, C. G. 1991. Steroids: developmental continuum between mother and offspring. In A. Scott, J. Sumpter, D. Kime & M. Rolfe, eds. *The Fourth International Symposium on the Reproductive Physiology of Fish*, pp. 256-258. Sheffield: FishSymp.

Schwarz, I. M. & Rogers, L. J. 1992. Testosterone: a role in the development of brain asymmetry in the chick. *Neuroscience Letters*, **146**, 167-70.

Sloman, K. A. 2010. Exposure of ova to cortisol pre-fertilisation affects subsequent behaviour and physiology of brown trout. *Hormones and Behavior*, **58**, 433-439.

Smith, L. L. & Hines, M. 2000. Language lateralization and handedness in women prenatally exposed to diethylstilbestrol (DES). *Psychoneuroendocrinology*, **25**, 497–512.

Sovrano, V. & Andrew, R. 2006. Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behavioural Brain Research*, **167**, 226–231.

Sovrano, V., Bisazza, A. & Vallortigara, G. 2001. Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiology & Behavior*, **74**, 237-44.

Sovrano, V., Rainoldi, C., Bisazza, A. & Vallortigara, G. 1999. Roots of brain specializations: preferential left-eye use during mirror image inspection in six species of teleost fish. *Behavioral Brain Research*, **106**, 175-180.

Tan, U. 1991. Serum testosterone levels in male and female subjects with standard and anomalous dominance. *International Journal of Neuroscience*, **58**, 211–214.

Vallortigara, G. & Bisazza, A. 2002. How ancient is brain lateralization. In L.J. Rogers & R.J. Andrew, eds. *Compartive Vertebrate Lateralization*, pp. 9-69. Cambridge: Cambridge University Press.

Vallortigara, G., Chiandetti, C. & Sovrano, V. 2011 Brain asymmetry (animal). *Wiley Interdisciplinary Reviews: Cognitive Science*, **2**, 146-157.

Witelson, S. F. & Nowakowski, R. S. 1991. Left out axons make men right: a hypothesis for the origin of handedness and functional asymmetry. *Neuropsychologia*, **4**, 327-333.

4

Sex-specific effects of postnatal testosterone on lateralization in cichlid fish

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Abstract

Lateralization is a fundamental principle in the organization of brain and behaviour in humans and nonhuman animals. To what extent lateralization is, in addition to genetic factors, under the influence of testosterone, which would also explain sex differences in laterality, is the topic of a long-standing debate. This debate is partly hampered by confusion between organizational and activating effects of testosterone. Here we focused on activating effects, less often studied than organizational effects. Studies on humans have shown ambiguous results and few experimental studies on animals have been conducted. We studied *Aequidens rivulatus*, a cichlid species in which lateralization of visually guided behaviour has been demonstrated and related to aggressiveness. After treatment with testosterone, lateralization was tested in a rotational preference task where fish had to face a predator. Testosterone induced significant lateralization at the population level. Testosterone-treated fish watched a predator preferentially with the right eye, which is in line with the literature on population-level lateralization of predator viewing in fish. It has been suggested that species differences in laterality of predator escape are related to sociality and we speculate that, within species, testosterone may affect lateralization in relation to a change in sociality. Among the fish of known sex, only males reacted to the treatment; a greater responsiveness of males may explain sex differences in lateralization found in many animal species. This may be caused by sex-specific sensitivity to androgens, perhaps in the habenular area of the brain.

Introduction

Cerebral lateralization, the asymmetric distribution of functions over the two hemispheres, is a widespread phenomenon among vertebrates and results in lateralization of behaviour. Several studies have shown that males show a different lateralization pattern to females (humans: Hiscock *et al.* 1994; Wisniewski 1998; birds: Adret & Rogers 1989; fish: Brown *et al.* 2007; Reddon & Hurd 2008; reviewed in Pfannkuche *et al.* 2009) and some authors have suggested that this difference arises because of differences in exposure to testosterone (Wisniewski 1998), either prenatally or later in life, which has organizational (Schwarz & Rogers 1992; Rogers & Rajendra 1993; Sanders *et al.* 2002) or activating effects (Diamond 1991; Sanders *et al.* 2002) on lateralization.

Most attention so far has focused on the potential organizational nature of pre- and perinatal testosterone on lateralization (reviewed in Pfannkuche *et al.* 2009). However, activating effects may be equally relevant, for two reasons. First, some of the organizational effects of testosterone on brain lateralization may come about because of organizational effects on sex-specific production and sensitivity to androgens later in life. Second, organisms need to make constant adjustments to changes in their internal state and external environment, which may facilitate changes in lateralization during their lifetime. Since it has been suggested that lateralization at the population level is especially adapted for social behaviour (Vallortigara & Rogers 2005), and many aspects of social behaviour are under the influence of androgens, these hormones may be involved in inducing changes in lateralization during the animal's lifetime. Unfortunately, the activating effects of testosterone have been studied far less extensively than organizational effects and hypotheses about how testosterone affects lateralization in the adult brain are lacking.

It is well known that testosterone production is sensitive to diurnal rhythm (Plymate *et al.* 1989; Dabbs 1990), seasonal rhythm (Dabbs 1990; Meriggiola *et al.* 1996; Garde *et al.* 2000) and social cues (Oliveira 2004), which facilitates investigating the relationship between testosterone levels within their natural range and performance of lateralized functions, such as spatial skills. These studies suggest that testosterone suppresses spatial skills and therefore perhaps lateralization (Gouchie & Kimura 1991; Kimura & Toussaint 1991; Moffat & Hampson 1996). However, these studies are correlative and causal relationships are as yet unclear. Contrary to the correlative studies, experimental studies in humans, in which older males or female-to-male transsexuals received testosterone therapy, indicate that testosterone increases scores on spatial cognition (Janowsky *et al.* 1994; Slabbekoorn *et al.* 1999; van Goozen *et al.* 2002; Gooren & Giltay 2008; but see Sommer *et al.* 2008; reviewed in Cherrier 2009).

Spatial ability as such, however, is not a direct measure of lateralization. A few studies have analysed the relation between circulating levels of testosterone in human adults and handedness, a more direct measurement of lateralization. Results from these studies are, however, ambiguous too: Tan (1991) found higher levels of circulating testosterone in left-handers, ambidexters and right-handers with familial sinistrality than in right-handers, whereas Gadea (2003) and Moffat & Hampson (1996) found lower levels in left-handers. Other studies found no relation between testosterone and handedness (Moffat

& Hampson 2000; Beaton *et al.* 2010). All of these studies assumed that individual differences in testosterone levels in adulthood reflect organizational effects of the hormone early in life. However, it is equally likely that these studies actually tested activating effects of testosterone.

Clearly, results of studies on the activating effects of androgens on human lateralization are ambiguous and call for experiments to be conducted on a random, nonpathological sample, representative of the population, using a direct measure of lateralization. Obviously, such experiments are more attainable in nonhuman animals than in humans, but are rare. Rogers *et al.* (1985) found that chickens, *Gallus gallus domesticus*, treated with testosterone early in life showed increased levels of attack when using their left eye but not when using their right eye, but whether this was an organizational or activating effect remains unclear. Del Rio-Portilla *et al.* (1997) found that in rats, *Rattus norvegicus*, gonadectomized after puberty, gonadal steroids were necessary to maintain sex differences in asymmetry shown in an EEG and a correlational study in primates showed that testosterone levels during adolescence were related to hand preference (Westergaard *et al.* 2000).

The evidence that testosterone has activating effects on lateralization is supported by indirect evidence from a recent study on cichlid fish. Adult fish showed a sex difference in lateralization, which correlated with their level of aggressiveness (Reddon & Hurd 2008). As aggressiveness and testosterone levels are correlated in fish (Munro & Pitcher 1985; Higby *et al.* 1991) the results suggest that testosterone activates sex differences in lateralization.

We experimentally tested the effect of testosterone on lateralization in male and female cichlid fish, expecting to find a similar interaction effect between sex and hormone treatment as Reddon & Hurd (2008) found between sex and aggressiveness. Fish have laterally placed eyes with small overlapping visual fields, which facilitates behavioural testing, easily revealing the lateralization pattern of the individual fish. In fish, predator inspection and predator viewing are lateralized (Bisazza & Vallortigara 1997; Bisazza *et al.* 1999; Brown *et al.* 2004): the fish preferentially inspect and view the predator using the right eye. Hence, we used predator viewing to investigate the effects of postnatal testosterone on the strength and direction of lateralization.

Methods

Subjects and Housing

Aequidens rivulatus (a schooling, sexually dimorphic, substrate-breeding cichlid from Ecuador and Peru; Stawikowski & Werner 1998) were bred at our laboratory. These fish, like most cichlids, show elaborate display behaviour during agonistic and sexual interactions and are the subjects of a long-term study of behavioural lateralization in our department. Fish were reared in large stock aquaria (100 x 50 cm and 50 cm high), of which the back and sides were covered with black plastic, and fed dry fish flakes daily (TetraMin Tropical Fish Flakes). *Astronotus ocellatus*, a South American predatory fish, were obtained from a local pet store. They were held in pairs in 200-litre tanks and were

kept in the laboratory after the study. They were fed dry fish flakes daily and additionally received frozen shrimp weekly. Fish were held in recirculating water filtration systems at 24 ±2 °C and on a 12:12 h light:dark cycle.

Hormone Treatment

We used fish at 6 months old, the age at which they are about to become sexually mature. We used this age for two reasons. First, this is long after the period of sexual differentiation in cichlid fish (Pandolfi *et al.* 2006), confirmed in this species in our laboratory (AFH Ros & TGG Groothuis, unpublished data), so that exposure to testosterone was unlikely to have organizational effects. Second, the fish were not yet sexually active and did not show the nuptial sexually dimorphic coloration. Hence, endogenous testosterone levels in males were not yet elevated, enabling us to use control fish without the need for castration, which may influence the physiology of the animal dramatically.

At the start of the experiment, 120 fish (standard length: mean + SD = 26.79 +· 3.49 mm) were distributed equally over the 12 tanks (35 x 24 cm and 24 cm high), half of which contained methyl-testosterone (Sigma), 0.5 mg dissolved in 0.0125 ml ethanol per litre water (a concentration shown to induce nuptial coloration and adult-like aggressive and sexual behaviour in *A. rivulatus*: Groothuis 1993), and half of which contained the solvent only, 0.0125 ml ethanol per litre water. Twice a week, we removed most of the water, leaving only a small layer, and replaced it with clean water, followed by renewal of the hormone treatment. Treatment lasted until the behavioural test started. For logistical reasons, only a limited number of fish could be tested each day, resulting in a variable treatment period of 8-12 weeks. This period of exposure to treatment was chosen based on a previous study (AFH Ros & TGG Groothuis, unpublished data) showing that juvenile fish show adult coloration and adult-like agonistic and courtship behaviour after this period of testosterone treatment and that the effects on both coloration and behaviour disappear after cessation of treatment.

Lateralization Test

We used a rotational preference test to investigate the lateralization of the fish. The apparatus consisted of a circular tank (diameter 30 cm) with a Plexiglas cylinder (diameter 12.5 cm) containing a predator (*A. ocellatus*) in the middle. To avoid possible lateralized interactions between the predator and the subject fish, the predator was restrained from moving by means of two Plexiglas plates. The tank was filled with 15 cm of water and was lit from above with a 60 W light bulb in the middle. Two sets of apparatus were used and placed in a completely darkened room. Before testing, the subject fish was placed in a small opaque tube, which was positioned anterior to the predator, in the darkened apparatus. After a 2 min acclimatization period, the light was switched on, the small opaque tube was gently removed from the tank and behaviour was video recorded for 15 min. Four adult *A. ocellatus* (standard length: mean + SD = 9.88 + 0.47 cm) were used as predator stimuli. The recordings were analysed using the software program The Observer (Noldus Information Technology, Wageningen, The Netherlands). Time spent swimming in a clockwise or anticlockwise position was computed. We excluded from analysis the time fish spent turning to change their viewing position or trying to escape from the apparatus (facing the outer wall). Following the literature, we calculated a

laterality index using the formula (time anticlockwise - time clockwise)/(time anticlockwise + time clock-wise) x 100. Positive values thus indicate more left-eye usage, whereas negative values indicate more right-eye usage when observing a predator. To investigate the strength of lateralization, independently of the direction, the absolute values of the laterality index were also computed.

Sex Determination

After euthanasia (see Ethical Note), the fish were weighed (to the nearest 0.1 g), measured (standard length to the nearest 1 mm) and decapitated. Fish were immediately frozen at -80 °C. At the time of histology the tail and epaxial musculature were removed and transverse serial sections were cut at 7e10 mm, stained with haematoxylin-eosin Y and examined under a light microscope. We scored the presence or absence of ovarian or testicular tissue. Hermaphrodite fish were not found.

Statistics

The laterality index and the log-transformed absolute laterality index were normally distributed and the variances of the dependent variables were not significantly different across groups. The laterality index and absolute laterality index were analysed using hierarchical linear models in the software program MLwiN 2.02 (Rasbash *et al.* 2005) to accommodate the hierarchical structure of our data set. We created two levels, tank being the highest and fish identity the lowest level. We fitted the models with treatment, sex and their interaction effect as categorical predictive factors. The models were estimated using restricted iterative generalized least squares (RIGLS). We tested the significance of the fixed factors using the Wald statistic, which follows a chi-square distribution.

To test whether groups showed a population bias of lateralization, we conducted one-sample t tests using the software package SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.). In addition, to investigate whether differences in duration of exposure to testosterone affected the laterality index, a linear regression analysis was performed using SPSS 16.0. All tests were two tailed and significance levels were set to $a < 0.05$.

Ethical Note

The testosterone or control treatment to which the experimental fish were exposed did not have any notable effects on their welfare. No adverse effects of testosterone were seen on survival or growth. Fish were killed by an overdose of MS-222 (1 g/litre) buffered with sodium bicarbonate. Opercular movement ceased within 1 min and fish were left in the solution additionally for at least 10 min. We used MS-222 in more than five times the anaesthetic solution (150 mg/litre for induction in cichlids, Neiffer & Stamper 2009) as recommended by the American Veterinary Medical Association (2007).

The predators, being hand tame and very calm in our tanks, were restrained during the behavioural trials by means of two Plexiglas plates to prevent any lateralized interactions between the predator and the subject fish. These plates were loosely placed in such a manner that the fish could still move but could not turn. The predators showed tonic immobility when first placed in this position, but after approximately 2 min showed a normal posture and no changes to a darker colour (a sign of stress in many cichlid fish) were noted. Individual *A. ocellatus* were used for a maximum time of 1 h and reused after

a 47 h period of recovery in its home tank. All experimental protocols were approved by the ethical committee for animal research of the University of Groningen.

Results

Consistent with our expectation, fish treated with testosterone showed a population bias to look at a predator with the right eye, thus using the left hemisphere (one-sample t test: $t_{19} = -2.268$, $p = 0.035$), whereas in the control treatment no such bias was found (one-sample t test: $t_{18} = 0.181$, $p = 0.858$; figure 1a). Testosterone treatment did not affect the absolute value of the laterality index (Wald $\chi^2_1 = 0.071$, $p = 0.790$; figure 1b).

Histological examination revealed 19 females (nine in control group, 10 in testosterone group), 10 males (five in each group) and 10 fish of which the sex could not be determined (five in each group) as no gonadal tissue was yet apparent. Testosterone affected the laterality index of males differently to that of females and this interaction effect between treatment and sex was significant (Wald $\chi^2_3 = 9.234$, $p = 0.026$; figure 2a). Post hoc tests showed that testosterone-treated males differed significantly from control males (Wald $\chi^2_1 = 5.427$, $p = 0.020$). No other post hoc tests were significant. Testosterone treatment did not affect the absolute value of the laterality index of sex-determined fish (interaction treatment*sex: Wald $\chi^2_3 = 2.180$, $p = 0.536$; treatment: Wald $\chi^2_1 = 0.066$, $p = 0.797$; sex: Wald $\chi^2_1 = 2.154$, $p = 0.142$; figure 2b).

Within the testosterone-treated group, there was no effect of the variation in duration of testosterone treatment on the laterality index ($\beta = -0.19$, $t_{17} = -0.836$, $p = 0.414$, $R^2 = 0.037$).

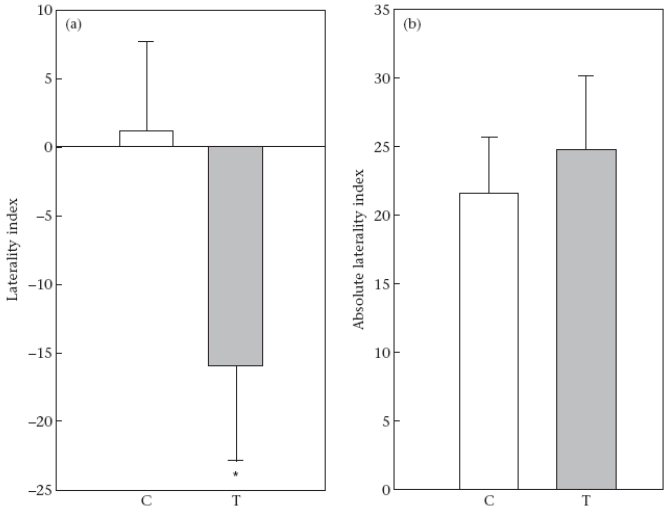

Figure 1 • (a) Laterality index of the rotational test in the control (C; white bars, N = 19) and testosterone treatment (T; grey bars, N = 20) groups. Positive values indicate more left-eye usage, negative values indicate more right-eye usage. (b) Absolute laterality index of the rotational test in the control and testosterone treatment groups. Means are shown + SEs. *P < 0.05.

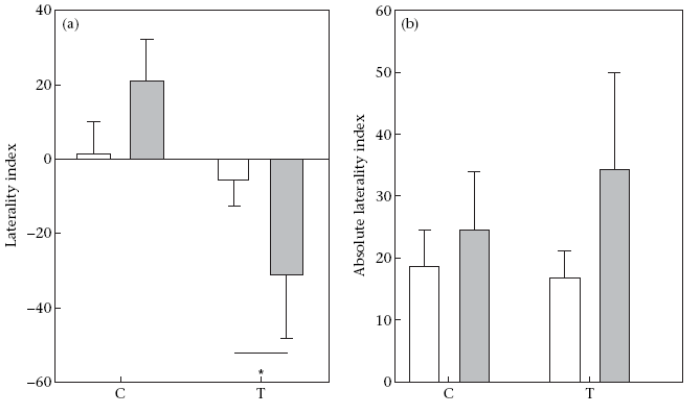

Figure 2 • (a) Laterality index of the rotational test for females (white bars, Ncontrol = 9, Ntestosterone = 10) and males (grey bars, Ncontrol = 5, Ntestosterone = 5) in the control (C) and testosterone treatment (T) groups. (b) Absolute laterality index of the rotational test for females and males in the control and testosterone treatment groups. Means are shown + SEs. *P < 0.05.

Discussion

We tested the effect of testosterone on visually guided behavioural lateralization in fish just before sexual maturity and found a clear effect in males, but not in females. In addition, testosterone induced lateralization at the level of the population. These fish preferentially viewed the predator with the right eye, thus using the left hemisphere. These findings are in line with the literature and our expectations. The direction of lateralization we found in fish treated with testosterone is in accordance with results obtained from similar tests of several fish species (Bisazza & Vallortigara 1997; Bisazza *et al*. 1999). Studies have shown that a bias to turn left, thus fixating on the predator with the right eye, occurs when fish are escaping from a predator (Cantalupo *et al*. 1995; Lippolis *et al*. 2009). A bias in the direction of the escape response within fish shoals is thought to be adaptive to a certain degree as it hampers the predator's attempts to catch its prey owing to a dilution effect in shoaling animals (Vallortigara & Rogers 2005).

This intraspecific variation in eye use resulting from the hormone treatment may be related to the explanation for inter-specific variation in eye use that is based on interspecific variation in the tendency to shoal (Bisazza *et al*. 2000), which may also vary with predation pressure and other environmental variables (Brown & Warburton 1997). In shoaling species, individuals must be able to monitor both predators and shoalmates simultaneously. Selection for such lateralization might be lacking in nonshoaling fish, but present in shoaling fish. It is intriguing that lateralization at the level of the population is seen as an adaptation for social behaviour (Vallortigara & Rogers 2005), that many social behaviours are under the influence of testosterone (Nelson 2005), and that we found an effect of testosterone on lateralization. Perhaps within species, testosterone induces a change in life history stage from being more solitary to becoming more social, inducing fish to come together on the breeding grounds, making synchronous escape movements away from predators beneficial.

Testosterone had different effects on males and females. This sex effect highlights the importance of studying lateralization with respect to the sex of the animal. Reddon & Hurd (2008) found an interaction effect between aggressiveness and sex on lateralization similar to our findings. Testosterone may be the underlying mechanism causing this result, as testosterone levels and aggressiveness are positively related in fish (Munro & Pitcher 1985; Higby *et al.* 1991). The finding that males were more sensitive to testosterone than females is consistent with other studies in many vertebrates and probably caused by the early organizational effects of steroids on sexual differentiation, for example by its effect on the synthesis of androgen receptors (Nelson 2005). Our results open the possibility that behavioural lateralization is partly induced by sex-specific lateralization of androgen receptors but as yet this has received no attention in the literature. Furthermore, testosterone is known to have sex-specific effects on the structural asymmetry of the habenular nucleus in the vertebrate brain: testosterone treatment changed the asymmetry of female chickens to that of males when applied soon after hatching (Gurusinghe *et al.* 1986). This nucleus is the best-known example of brain asymmetry (Concha & Wilson 2001). In frogs, both males and females show asymmetries of the dorsal habenula, and these asymmetries are more pronounced in spring, the mating season, than in winter, indicating a modulating role for activating effects of testosterone on the habenula (Kemali *et al.* 1990).

All the effects of testosterone that we found concerned the direction and not the strength of lateralization. Assuming that testosterone regulates aggression, our results are therefore inconsistent with the finding that sex and aggressiveness interact in affecting the strength of lateralization in another cichlid species (Reddon & Hurd 2008). However, the translation of their results to ours may be too simplistic, as aggression is not only a reflection of levels of testosterone and testosterone does not only regulate aggression.

In conclusion, the predominant genetic models concerning lateralization are limited in their explanatory power and leave scope for environmental factors such as gonadal steroids to affect lateralization patterns (Schaafsma *et al.* 2009/chapter 2, this thesis). However, this potential hormonal effect is still heavily disputed. Although we cannot entirely rule out the possibility that the effect of testosterone treatment that we found might have been both activating and organizational, our study showed that lateralization is a plastic trait and that testosterone affects lateralization well after the pre-and perinatal period. Males were more responsive to exogenous testosterone than females in the effect on lateralization, and this difference in responsiveness may explain sex differences in lateralization that have been demonstrated in several species. Our results therefore provide a model to analyse the activating effect of testosterone in neurobiology, an enterprise so far receiving much more attention for the study of organizational effects of the hormone on lateralization.

Acknowledgements

We thank Ronald Booms (University of Wageningen) and Jan Keijser (University of Groningen) for their help with determining the sex of the fish and Floor Bouwman for help with the behavioural tests. The study was supported by the Commission of the European Communities within the framework of the project EDCBNL (Evolution and Development of Cognitive, Behavioural and Neural Lateralisation).

References

Adret, P. & Rogers, L. 1989. Sex differences in the visual projections of young chicks: a quantitative study of the thalamofugal pathway. *Brain Research*, **478**, 59-73.

American Veterinary Medical Association 2007. Guidelines on Euthanasia. http:// www.avma.org/issues/animal_welfare/euthanasia.pdf.

Beaton, A. A., Rudling, N., Kissling, C., Taurines, R. & Thome, J. 2010. Digit ratio (2D:4D), salivary testosterone, and handedness. *Laterality*, **15**, 1-20.

Bisazza, A. & Vallortigara, G. 1997. Rotational swimming preferences in mosquitofish: evidence for brain lateralization? *Physiology & Behavior*, **62**, 1405-1407.

Bisazza, A., De santi, A. & Vallortigara, G. 1999. Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Animal Behaviour*, **57**, 1145-1149.

Bisazza, A., Cantalupo, C., Capocchiano, M. & Vallortigara, G. 2000. Population lateralisation and social behaviour: a study with 16 species of fish. *Laterality*, **5**, 269-284.

Brown, C. & Warburton, K. 1997. Predator recognition and anti-predator responses in the rainbowfish Melanotaenia eachamensis. *Behavioral Ecology and Sociobiology*, **41**, 61-68.

Brown, C., Gardner, C. & Braithwaite, V. 2004. Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proceedings of the Royal Society B*, **S271**, S455-S457.

Brown, C., Western, J. & Braithwaite, V. 2007. The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour*, **74**, 231-238.

Cantalupo, C., Bisazza, A. & Vallortigara, G. 1995. Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia*, **33**, 1637-1646.

Cherrier, M. 2009. Testosterone effects on cognition in health and disease. *Frontiers of Hormone Research*, **37**, 150.

Concha, M. L. & Wilson, S. 2001. Asymmetry in the epithalamus of vertebrates. *Journal of Anatomy*, **199**, 63-84.

Dabbs, J. 1990. Salivary testosterone measurements: reliability across hours, days, and weeks. *Physiology & Behavior*, **48**, 83-86.

Del Rio-Portilla, I., Ugalde, E., Juarez, J., Roldan, A. & Corsi-Cabrera, M. 1997. Sex differences in EEG in adult gonadectomized rats before and after hormonal treatment. *Psychoneuroendocrinology*, **22**, 627-642.

Diamond, M. 1991. Hormonal effects on the development of cerebral lateralization.*Psychoneuroendocrinology*, **16**, 121-129.

Gadea, M. 2003. Salivary testosterone is related to both handedness and degree of linguistic lateralization in normal women. *Psychoneuroendocrinology*, **28**, 274-287.

Garde, A. H., Hansen, A. M., Skovgaard, L. T. & Christensen, J. M. 2000. Seasonal and biological variation of blood concentrations of total cholesterol, dehydroepiandrosterone sulfate, hemoglobin A(1c), IgA, prolactin, and free testosterone in healthy women. *Clinical Chemistry*, **46**, 551-559.

Gooren, L. & Giltay, E. 2008. Review of studies of androgen treatment of female-to-male transsexuals: effects and risks of administration of androgens to females. *Journal of Sexual Medicine*, **5**, 765-776.

van Goozen, S., Slabbekoorn, D. & Gooren, L. 2002. Organizing and activating effects of sex hormones in homosexual transsexuals. *Behavioral Neuroscience*, **116**, 982-988.

Gouchie, C. & Kimura, D. 1991. The relationship between testosterone levels and cognitive-ability patterns. *Psychoneuroendocrinology*, **16**, 323-334.

Groothuis, T. 1993. The ontogeny of social displays e form development, form fixation, and change in context. *Advances in the Study of Behavior*, **22**, 269-322.

Gurusinghe, C. J., Zappia, J. & Ehrlich, D. 1986. The influence of testosterone on the sex-dependent structural asymmetry of the medial habenular nucleus in the chicken. *Journal of Comparative Neurology*, **253**, 153-162.

Higby, M., Beulig, A. & Dwyer, J. 1991. Exogenous testosterone and social experience each enhance the development of aggressive behavior in *Cyprinodon variegatus*. *Aggressive Behavior*, **17**, 229-239.

Hiscock, M., Inch, R. & Jacek, C. 1994. Is there a sex difference in human laterality? 1. An exhaustive survey of auditory laterality studies from 6 neuropsychology journals. *Journal of Clinical and Experimental Neuropsychology*, **16**, 423-435.

Janowsky, J., Oviatt, S. & Orwoll, E. 1994. Testosterone influences spatial cognition in older men. *Behavioral Neuroscience*, **108**, 325-332.

Kemali, M., Guglielmotti, V. & Fiorino, L. 1990. The asymmetry of the habenular nuclei of female and male frogs in spring and in winter. *Brain Research*, **517**, 251.

Kimura, D. & Toussaint, C. 1991. Sex differences in cognitive function vary with the season. *Society for Neuroscience Abstracts*, **17**, 868.

Lippolis, G., Joss, J. M. & Rogers, L. J. 2009. Australian lungfish (*Neoceratodus forsteri*): a missing link in the evolution of complementary side biases for predator avoidance and prey capture. *Brain, Behavior and Evolution*, **73**, 295-303.

Meriggiola, M. C., Noonan, E. A., Paulsen, C. A. & Bremner, W. 1996. Annual patterns of luteinizing hormone, follicle stimulating hormone, testosterone and inhibin in normal men. *Human Reproduction*, **11**, 248-252.

Moffat, S. D. & Hampson, E. 1996. A curvilinear relationship between testosterone and spatial cognition in humans: possible influence of hand preference. *Psychoneuroendocrinology*, **21**, 323-337.

Moffat, S. D. & Hampson, E. 2000. Salivary testosterone concentrations in left-handers: an association with cerebral language lateralization? *Neuropsychology*, **14**, 71-81.

Munro, A. D. & Pitcher, T. J. 1985. Steroid hormones and agonistic behavior in a cichlid teleost, Aequidens pulcher. *Hormones and Behavior*, **19**, 353-371.

Neiffer, D. & Stamper, M. 2009. Fish sedation, analgesia, anesthesia, and euthanasia: considerations, methods, and types of drugs. *ILAR Journal*, **50**, 343-360.

Nelson, R. 2005. An Introduction to Behavioral Endocrinology. Sunderland, Massachusetts: Sinauer Associates.

Oliveira, R. 2004. Social modulation of androgens in vertebrates: mechanisms and function. *Advances in the Study of Behavior*, **34**, 165-239.

Pandolfi, M., Lo Nostro, F. L., Shimizu, A., Pozzi, A. G., Meijide, F. J., Vazquez, G. R. & Maggese, M. C. 2006. Identification of immunoreactive FSH and LH cells in the cichlid fish *Cichlasoma dimerus* during the ontogeny and sexual differentiation. A*natomy and Embryology*, **211**, 355-365.

Pfannkuche, K. A., Bouma, A. & Groothuis, T. G. 2009. Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species. *Philosophical Transactions of the Royal Society B*, **364**, 929-942.

Plymate, S., Tenover, J. S. & Bremner, W. 1989. Circadian variation binding in healthy in testosterone, sex hormone- and calculated globulin bound testosterone and elderly men. *Journal of Andrology*, **10**, 366-371.

Rasbash, J., Charlton, C., Browne, W. J., Healy, M. & Cameron, B. 2005. MLwiN. Version 2.02, Multilevel Models Project. London: Institute of Education, University of London. http:// www.cmm.bristol.ac.uk/MLwiN/

Reddon, A. R. & Hurd, P. L. 2008. Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters*, **4**, 338-340.

Rogers, L. J. & Rajendra, S. 1993. Modulation of the development of light-initiated asymmetry in chick thalamofugal visual projections by oestradiol. *Experimental Brain Research*, **93**, 89-94.

Rogers, L. J., Zappia, J. & Bullock, S. P. 1985. Testosterone and eye-brain asymmetry for copulation in chickens. *Experientia*, **41**, 1447-1449.

Sanders, G., Sjodin, M. & de Chastelaine, M. 2002. On the elusive nature of sex differences in cognition: hormonal influences contributing to within-sex variation. *Archives of Sexual Behavior*, **31**, 145-152.

Schaafsma, S. M., Riedstra, B. J., Pfannkuche, K. A., Bouma, A. & Groothuis, T. G. 2009. Epigenesis of behavioural lateralization in humans and other animals. *Philosophical Transactions of the Royal Society B*, **364**, 915-927.

Schwarz, I. M. & Rogers, L. J. 1992. Testosterone: a role in the development of brain asymmetry in the chick. *Neuroscience Letters*, **146**, 167-170.

Slabbekoorn, D., van Goozen, S. H., Megens, J., Gooren, L. & Cohen-Kettenis, P. T. 1999. Activating effects of cross-sex hormones on cognitive functioning: a study of short-term and long-term hormone effects in transsexuals. *Psychoneuroendocrinology*, **24**, 423-447.

Sommer, I. E., Cohen-Kettenis, P. T., van Raalten, T., Vd Veer, A. J., Ramsey, L. E., Gooren, L., Kahn, R. S. & Ramsey, N. F. 2008. Effects of cross-sex hormones on cerebral activation during language and mental rotation: an fMRI study in transsexuals. *European Neuropsychopharmacology*, **18**, 215-221.

Stawikowski, R. & Werner, U. 1998. Die Buntbarsche Amerikas. Vol. 1. Stuttgart: E. Ulmer.

Tan, U. 1991. Serum testosterone levels in male and female subjects with standard and anomalous dominance. *International Journal of Neuroscience*, **58**, 211-214.

Vallortigara, G. & Rogers, L. J. 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences*, **28**, 575-589.

Westergaard, G. C., Chavanne, T. J., Lussier, I. D., Suomi, S. J. & Higley, J. D. 2000. Hormonal correlates of hand preference in free-ranging primates. *Neuropsychopharmacology*, **23**, 502-507.

Wisniewski, A. B. 1998. Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. *Psychoneuroendocrinology*, **23**, 519-547.

5

Plasticity of lateralization: schooling predicts hand preference but not hand skill asymmetry in a nonindustrial society

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Abstract

Considerable variation in the frequency of left-handedness between cultures has been reported, ranging from 0.5 to 24%. This variation in hand preference may have evolved under natural or cultural selection. It has been suggested that schooling affects handedness but as in most human societies only a selected and minor part of the population does not attend school this is difficult to test. We investigated to what extent schooling affects both hand preference and asymmetry in hand skill in a non-industrial population in the highlands of New Guinea. This provided unique opportunities because of the relatively recent establishment of a primary school in this population still living the non-industrial traditional life in which handedness may have evolved. We interviewed 620 inhabitants (aged 5 – 70y) to collect demographic data and school history, tested hand preference on 10 ecologically relevant activities, and measured performance of each hand on three tasks (pegboard, grip force, ball throwing). Schooling was associated with preference, as schooled individuals were more likely to be extremely right-handed and less likely to be strongly right-handed, but not with asymmetry of hand skill (controlled for sex and age), indicating developmental plasticity in hand preference. Developmental plasticity in hand preference but not skill asymmetry, and the weak correlations between hand preference and hand skill asymmetry indicate that they represent different aspects of brain lateralization. Furthermore, schooled individuals were overall faster in fine motor performance, had greater grip strength and greater throwing accuracy. This suggests that there is selection on the fitter part of the population to enter school. The weak correlations between hand preference and hand skill asymmetry leave room for moderating factors such as schooling, sex, and age to have a differential effect on hand preference and hand skill and each needs to be studied in its own right.

Introduction

Individuals with right hand preference for a representative set of common manual tasks being the large majority is a characteristic of all human populations, and there is evidence that the relatively low frequency of left handedness exists over a long period of human evolution, as detailed analyses of cave drawings and tool production and use suggest (Bradshaw & Rogers 1996). Considerable variation in left-handedness between cultures, ranging from 0.5 to 24%, has been reported (Perelle & Ehrman 1994; Marchant & McGrew 1998; Faurie *et al.* 2005; Faurie & Raymond 2005), but the cause of this variation remains unclear. The variation has been explained as a result of variation in natural selection pressure on winning fights (Raymond *et al.* 1996; but see Schaafsma *et al.* 2011/ chapter 6, this thesis) in which left-handers would have an advantage as long as they are in the minority. Populations in which fighting is important are thus expected to hold a higher frequency of left-handers. Furthermore, variation has been explained to result from variation in cultural pressure against the use of the left hand for certain activities (writing, eating) (Perelle & Ehrman 1994; Holder & Kateeba 2004; Medland *et al.* 2004). Indeed, extreme right-handedness for writing was found to be more frequent in cultures having many formal rules and extreme left-handedness more frequent in cultures with few (Medland *et al.* 2004). However, as Marchant and McGrew (1998) pointed out, most studies in this field use a limited set of measures to assess hand use, and differ in age ranges of their sample, a factor known to influence lateralization (Bishop 1990; Gilbert & Wysocki 1992; Roy *et al.* 2003). Moreover, different studies used different criteria for classification of handedness, ranging from only the distinction between left and right to a graded system of direction and strength in terms of ambidexter, weak, strong and extreme. These limitations may explain part of the reported variations within and between cultures. Moderating factors such as sex and age affect handedness (sex: Papadatou-Pastou *et al.* 2008; age: Bishop 1990; Gilbert & Wysocki 1992), the latter indicating developmental plasticity of lateralization. Another factor that may influence lateralization is schooling. Connolly and Bishop (1992) investigated the association between schooling and handedness in a Papuan population. This population is ideal for investigating this association as a substantial part of the population did not attend school. Furthermore, associations found in this non-industrial population may be of special relevance as the individuals in this population live a traditional life in which handedness may have evolved. These authors assessed hand preference of children and adolescents in a Papuan population by observing subjects performing a set of nine actions with typically 'Western' objects such as dealing cards and using scissors. Against their predictions, there was a trend for those who had no schooling to be more right-handed for a task with a pencil (to fill in a small white square) than those who had received schooling. Schooling had no effect on handedness in other tasks. However, due to unfamiliarity with some of the actions these measures may not represent the subject's true handedness (see Bryden *et al.* 1993). To investigate whether schooling predicts handedness on measures that are of ecological relevance, we measured handedness in a similar Papuan non-industrial population, the Eipo, using activities that are common in the daily life of the individuals. This population offered the unique chance to study the effect of schooling as about 30% of this population spent some time in primary school. Investigating this population was further motivated

by the virtual lack of specialized craftsmen for making tools and ornaments in this population. The latter is important because tools made by craftsmen may impose handedness onto the user of the tool, thus exerting pressure on skill development of a single hand promoting mostly right-hand preference, as is the case in industrial societies.

Hand preference is generally explained as the expression of brain lateralization of fine motor function at the behavioral level. However, our limbs, being the 'tools' with which we physically interact with the environment, are symmetrical and our neuromotor system for voluntary action is symmetrical to a large extent too. This enables us to perform tasks with either limb but there are subtle differences in skill between the left and right limb. Similar to hand preference, this difference in skill between the hands is interpreted as an expression of cerebral lateralization of fine motor function at the behavioral level. The resulting difference in skill between the hands seems to be only weakly related with hand preference (Borod *et al.* 1984; Triggs *et al.* 2000; Doyen *et al.* 2008) and is task dependent (Steenhuis 1999; Corey *et al.* 2001; Cavill & Bryden 2003). Therefore it is important to study both preference and skill asymmetry, each with a representative set of tasks. Moreover, although it is assumed that hand preference may have evolved under natural selection, this may be even more likely so for hand skill. Greater overall hand performance and asymmetry of skill may enable the subject to better deal with the environment and increase chances for survival, thereby thus increasing its fitness. In addition, lateralization consists of two different aspects: direction and strength (Nettle 2003; Volkmann *et al.* 2011). Therefore, we investigated the effect of schooling on and the relation between hand preference, overall hand performance and hand skill asymmetry, both for strength and direction.

As it is known that there are small but consistent sex differences in handedness across cultures (Papadatou-Pastou *et al.* 2008) sex is included as a moderator. Age is included as a covariate because handedness can change with age (2.5-8.5y - Bishop 1990; 10-86y - Roy *et al.* 2003; Gilbert & Wysocki 1992), while also schooling can change with age as the youngest and oldest parts of the population had less or no opportunity to participate in schooling. With respect to schooling we predict that subjects with schooling will be more right-handed than those without (Perelle & Ehrman 1994; Holder & Kateeba 2004; but see Connolly & Bishop 1992). Additionally, as schooling beholds specific and intense training in fine motor control, we expect that schooled individuals will show stronger asymmetry in hand skill, especially in fine motor skill, than individuals without schooling.

We used three hand skill tasks that measure speed (in a pegboard task), accuracy (in a ball throwing task) and strength of each hand. We explored how these three measures relate to each other and to direction and strength of hand preference in this population. As a validation of the skill tasks overall hand skill is analyzed with the predictions that males will be stronger and better throwers than females. The pegboard will be validated with data in the literature. Our data concerning hand preference were validated before (Schaafsma *et al.* 2011/chapter 6, this thesis).

Method

Subjects and population

The population of this study are the Eipo people living in the upper part of a valley of the Eipo river (altitude >1500 m) in the central mountain range of New Guinea (Indonesia). In this isolated area, only accessible by light aircraft or by mountain trails, some 1600 people live spread over 3 villages and some small hamlets. They have their own language (*Eipo yupe*), and live a traditional life growing vegetables, sweet potatoes, tubers and fruits, with additional supply of protein rich food from hunting and recently also fish ponds. First brief contacts with non-Papuan people occurred in 1959 and 1968. From 1974 onwards German scientists regularly visited these villages (Schiefenhövel 1997; Ploeg 2004). Missionaries were active from 1977 to about 2000 and converted the larger part of the population to Christianity in 1981.

A primary school was built in 1981 and a part of the population, both children and adults, then went to school. No school fee is to be paid. In primary school they learn the Indonesian language, writing and other usual topics. Thus a portion of the population could speak and/or understand at least some Indonesian. Higher education is available in larger inland towns at a distance of at least 5 days walking, so children attending these schools were rarely present.

People did not know their age or that of their children. We estimated the age of the subjects using the technique of a calendar of events known to them (see Table 1) of which the year of occurrence was known to us. We used interpolation between these events using questions like: "Were you married when …?" and "Can you point to a child here who has the same age as you when …?" Additionally we used comparisons like "are you older (younger) than X?"; X being a person whose age we had accurately determined.

Our research was introduced in a speech during church service in each of the three villages. This speech in bahasa Indonesia was translated into the Eipo language by one of the local people. Three research assistants with appropriate knowledge of both languages assisted in the interviews and tasks. The research project was approved by the ethical

Table 1 • Calendar of events used in estimating the age of the adult subjects

Year		Event
1959	*	first contact – the French/Dutch expedition led by Gaisseau
1969	*	Indonesian parachutists with medical dr. Bondan land in the Eipo valley using flares
1974	*	arrival German scientific expedition; some scientists stayed for 18 months
1976	**	1976 - two earthquakes of magnitude 7.1 on June 25 and October 29
1981		school and missionary house built
1989		new church built
1995		arrival of missionary Janovski
1999		first fishing ponds
2005		health care center built

* from Schiefenhövel (1997)

** http://earthquake.usgs.gov/earthquakes/world/historical_country.php (retrieved 15 Oct. 2010)

committee of the Psychology Department of the University of Groningen, and subjects were recruited only on a voluntary basis with informed verbal consent.

In total 621 subjects participated, 289 males and 332 females, with an age range of 5 to 70 years, of which 235 were younger than 18 years; 400 (65%) had never attended primary school, and 186 (30%) completed at least one year in primary school. Individuals that did enter school, but attended less than one year were excluded from the analyses.

Interview

All interviews and tests were done by the first two Indonesian speaking authors, each always assisted by one of the three local assistants. In the interview that lasted about 30 minutes demographic information of the subject and its family was collected, including an estimation of the subject's age, self-reported hand preference and school history (none, years spent in preschool, primary school, secondary school, higher education). The interview was followed by a hand preference test and three hand performance tasks, in the following order.

Hand preference test and procedure

Hand preference was determined in a similar way as in the study of (Annett 1970) by observation of spontaneous hand use in a series of 12 unimanual and bimanual tasks that we adapted to the daily life of the Papuan population (see Table 2, Schaafsma *et al*. 2011/ chapter 6, this thesis). Subjects were seated on the ground or a low rock with their legs crossed (items 1-9) or stood upright with both hands free directly facing the observer who was positioned in a similar posture. The materials were presented to the participant in such a way that no cue was provided as to the hand to use by first placing objects symmetrically on a wooden board, then with two hands placed in front of the subject within reach (items 1- 6, 9) or presented to the subject by the testers holding the object(s) symmetrically with both hands (items 8, 10-12).

Table 2 • List of 12 activities used to determine hand preference (adapted from Annett, 1970, and Oldfield, 1971). Tasks 10 and 12 were not included in the hand preference index (see text).

Task nr	**Description of question and task**: *"Can you show me how you …*	**Materials**
1	sharpen this stick with this knife	handknife, stick (L 50cm)
2	take the stone and hit the pebble to crush it	pebble, handsize stone
3	draw a circle on the earth with this stick	stick (L 50cm)
4	pick up the small bead and hand it over	bead (Ø 2mm)
5	pick up the stone and the pole and hammer it in the earth with the stone	pole (45cm); handsize stone
6	pick up and eat a peanut	peeled peanut
7	chase an imaginable fly from your nose	
8	punch this bag	cloth bag (20x20 cm) filled with soft material hanging on short rope
9	throw away this little pebble	small pebble
10	use a bush knife	bush knife
11	use a digging stick	Stick (length 2m)
12	shoot this arrow with this bow	bow and arrow

Hand skill tests and procedure

Three tests were used that measured the speed and accuracy of peg placement, hand grip force, and the accuracy of throwing. Both hands were tested, the initial hand alternated over subjects. The three tests were administered in fixed order.

The Annett pegboard (Annett 1985 p. 208) was constructed of hardwood (L x W x H = 40 x 21 x 2.5 cm) with 2 rows of 10 holes (Ø 0.5 cm, depth 1.2 cm) each 3.75 cm apart and distance between the rows 17 cm. The ten steel pegs (Ø 0.45 cm, length 3 cm) are placed in one row. Subjects were seated on a low flat rock with a thin board (± 50 x 40 cm) on their lap. The pegboard was placed on this board, the row of pegs closest to the body at a distance of approximately 10 cm. Subjects held the pegboard with one hand, while performing the task with the other. Subjects were instructed to move the pegs one by one to the compatible location at the other row working from the side of the hand as fast as possible without dropping a peg. Each trial started with a practice trial moving 3 pegs forth and back. If the task had not properly been understood a second practice trial was given. Next, the subject was instructed to hold the first peg and to start after the tester had counted from 3 back to zero. The time to complete the task was measured with a stopwatch. If a peg was dropped the task was stopped, and the trial repeated. The Annett pegboard task has been used in many studies (e.g. in Papua: Connolly & Bishop 1992) and is a valid and reliable tool to measure hand skill asymmetry (Annett 1992).

Hand grip force was measured with a hand dynamometer (Bramshey BRSFU238) in a standard posture as follows: Subjects were seated with the thin board on their lap. The tester demonstrated the required arm and hand posture and briefly squeezed. Specifically it was demonstrated that the elbow should be close to but not against the side of the body, the lower arm approximately at an angle of 90 degrees forward and about halfway between pronation and supination, and the fingers straight at a 90º angle with the hand. Then the dynamometer was placed firmly in the 90º angle parallel to the fingers, the fingers folded over the handle, the thumb along the other side, with the dynamometer extending in line with the lower arm. The lower arm and dynamometer were not to touch the board, the other arm rested on the board. The subject was allowed to produce a light squeeze to 'get the feel' of the dynamometer. At the command 'squeeze' the subject was instructed to squeeze as hard as possible during 5 s. The dynamometer recorded the maximum force produced which was then copied on paper. If the posture deviated too much (i.e. the arm was stretched) or if the subject pushed the dynamometer hard on the board, or used help of the other hand, the trial was repeated for both hands. This was also done if the difference in force between the hands was unrealistic (criterion 20%). The few missing data were caused by subjects with a wound or malformation of one hand. The repeatability of this test was estimated from 38 randomly repeated trials. Cronbach's alpha was good with 0.896 for the asymmetry of hand skill index (SI) and 0.744 for absSI.

Accuracy of throwing was measured with a tennis ball and a target at a distance of 2.7 m for adults and 2m for children under 13y and the few elderly lacking force. The subjects stood facing the target hanging against a wall at 1.5 m height. The target had a white center area (Ø 7 cm; score 50 points) and 4 concentric black or white bands (width 2.8 cm each; scores: 40, 30, 20, 10 points respectively) on a black cloth (40 x 50 cm). The zero band was assumed to be the adjoining band of 2.8 cm width. Subjects were instructed to aim at

the center target. The score of each throw was observed from a position just behind and slightly aside the participant and noted on a scale of -50 to +50. If the ball landed outside the zero band the scores -50 to -10 were estimated by imagining the landing position mirrored around zero onto the target area. Occasional landing positions further away than -50 (i.e. more than 34 cm from the zero band) were scored -50. There were 5 trials per hand.

Analyses

Hand preference

During each of the 12 actions observed, left-hand use was scored as -1, right-hand use as +1, and the use of both hands or a change of hand during the unimanual task was scored as 0. In case of doubt whether the task had been properly understood, the score was recorded as missing. Shooting the bow was not included in the analyses because it was unfamiliar for the majority of the females. The total score over all 11 tests was calculated. Item - rest score correlations clearly indicated that the 'digging stick' showed much lower item-rest correlation (r= 0.093) than the other items (r = 0.51 to 0.90). Factor analysis indicated that the 10 remaining items load on one factor, with 69% of variance explained (compare to 71% reported by Corey *et al.* (2001) for a similar test in a US sample). A hand preference index (PI) representing hand preference based on the remaining ten items was calculated as the sum of scores divided by the number of scores, resulting in $-1 \leq PI \leq 1$. In line with some of the literature hand preference was also classified as extreme (PI = +1 or -1), strong ($-1 < PI \leq -0.8$ or $PI = +0.8 \leq PI < 1$), weak ($-.8 < PI \leq -0.5$ or $0.5 \leq PI < 0.8$), and ambidexter ($-.5 < PI < 0.5$). The absolute value of PI (absPI) representing the strength of hand preference independent of direction was also analyzed. Differences in distribution of PI between subgroups of the sample were tested with Kolmogorov-Smirnov Exact tests (2-tailed). Posthoc, confidence intervals for proportions were calculated for the differences between the distributions (Agresti & Coull 1998).

Mean hand skill and asymmetry of hand skill

As a measure of total task skill the mean score of the two hands was taken for each of the three tasks. The asymmetry between the hands was expressed as an asymmetry of hand skill index (SI) such that positive values indicate better performance of the right hand: $SIpegs=(L-R)/(R+L)$ and $SI =(R-L)/(R+L)$ for the other two tasks, with R and L representing the performance of the right and the left hand respectively. Absolute values of SI (absSI) represent the strength of relative asymmetry in performance between the hands. As the absolute values of the SI have left-skewed distributions the absSI values were transformed by $1/\ln(absSI+.001)$ to a normal distribution, the constant added to prevent dividing by zero. All analyses with these transformed variables yielded an identical set of significant statistical results to those with untransformed variables. Because interpretation of non-transformed variables is more transparent, we used the original absSI values in the analyses. A MANCOVA was used to analyze the effects of schooling (yes/no) and sex on the mean performance, SI and absSI of the three tasks, with sex as a moderator, followed by ANOVA's on separate tests scores. As age showed a quadratic relationship with mean skill and with skill asymmetry measures, age and age^2 were entered as a cova-

riate. Only the main effects were included in the models as we had no predictions for any interaction.

Relationships between hand preference and asymmetry of hand skill

The relationships between the three SI measures were tested by Pearson correlations; the relation between hand preference and hand skill asymmetry measures by Spearman rank correlations.

Results

Hand preference

Mean PI (range -1 to +1) was 0.88 and 97% of subjects had a positive PI, (right hand preference) and only 3% a negative PI (left hand preference; CI's respectively 94.1% – 99.2% and 1.8% – 4.6%). Mean self reported handedness was 0.92. Observed preference was concordant with self reported handedness in 95.6% of cases (2.4% report to be left-handed but score extreme right-handedness, 0.8% the other way around, 1% missing). The distribution of the hand preference index in subgroups of schooling by sex is shown in Table 3. First the effects of age and sex are reported as these may moderate the effect of schooling. Males were more often extreme right-handed (88.5% vs 82.5%) and less often weak to strong right-handed (7.3% vs 14.5%) than females (PI: Kz = 0.75; p = 0.046 ; absPI: Kz = 0.81, p = 0.023).

The effect of age on hand preference was studied between 3 age groups: children (5-17y, n = 242), younger adults (18-44y, n = 230) and older adults (45-70y, n = 148), representing more or less 3 generations. The distributions of PI did not differ between the two younger age groups (Kz = 0.38; p = 0.34), but did between the adult groups (Kz = 1.24; p = 0.001), and between the children and the older adult group (Kz = 1.55; p< 0.0001). The differences were due to fewer subjects with extreme right-handedness (PI = 1, CI's 86.1% - 93.6%, 81.4% - 91.2% and 67.4% - 80.0% respectively) and more with strong right-handedness (PI = 0.8,

Table 3 • Distribution of hand preference in a Papuan population, and in subgroups of sex and schooling

				Without schooling				With schooling			
		total sample		males		females		males		females	
PI class	**PI**	**freq.**	**%**	**freq.**	**%**	**freq.**	**%**	**freq.**	**%**	**freq.**	**%**
extreme L	-1	10	1.61	5	3.01	3	1.28	0	0	2	2.60
strong L	-0,8	4	0.65	3	1.81	0	0	1	0.93	0	0
weak L	-0,6	1	0.16	1	0.60	0	0	0	0	0	0
	-0,4	1	0.16	0	0	0	0	1	0.93	0	0
	-0,2	2	0.32	0	0	1	0.43	0	0	0	0
ambidexter	0	1	0.16	0	0	1	0.43	0	0	0	0
	0,2	2	0.32	1	0.60	1	0.43	0	0	0	0
	0,4	1	0.16	1	0.60	0	0	0	0	1	1.30
weak R	0,6	18	2.90	2	1.20	13	5.56	1	0.93	1	1.30
strong R	0,8	51	8.23	14	8.43	29	12.39	3	2.78	4	5.19
extreme R	1	529	85.32	139	83.73	186	79.49	102	94.44	69	89.61
total*		**620**	**100**	**166***	**100**	**234***	**100**	**108***	**100**	**77***	**100**

* differences in totals due to exclusion of 35 children with a few months to 1 year of primary school

CI's 2.8% - 7.0%, 4.9% - 13.0% and 10.6% - 21.0% respectively) in the older adult group. Similar effects were found for absPI (Kz = 0.47, p = 0.153; Kz = 1.25, p = 0.001 and Kz = 1.64, p < 0.0001, respectively).

The effect of schooling on hand preference was tested comparing the group that had at least one year of primary education (n = 185) with the group that never had education (n = 400). The groups differed on the PI (Kz = 1.26; p = 0.001). Subjects with schooling compared to those without were more often extreme right-handed (respective CI's 87.6% – 95.5% and 77.1% - 84.8%) and less often strong right-handed (respective CI's 1.7% - 7.8% and 8.1% - 14.2%) (see Figure 1). Similar differences were found for absPI (Kz = 1.15, p = 0.001). Given the effect of sex on the distribution the effect of schooling was compared within subgroups. The differences in distribution due to schooling within males (PI p = 0.009; absPI p = 0.045) and females (PI p = 0.065 ; absPI p = 0.039) were similar to those reported for the whole group. The moderating effect of age was not further explored because the oldest age group only had 17 subjects who had been to school, which is too few to compare subgroups, and implies that age cannot confound the effect of schooling on the distribution of hand preference in our sample.

Factors predicting mean hand skill

The means (and range) of mean hand skill of both hands on the three tasks was for pegboard 15.6 s (11.2 to 26.5 s); for grip force 23.7 kg (5.5 to 53.1 kg); and for ball throwing a score of 16.1 (-13.5 to +38). Performance on the pegboard is in the normal range (e.g. Annett 1985), and, as expected, males were stronger (26.8 vs. 20.8 kg) and better throwers (score 17.8 vs. 14.5), compared to females which validates these tasks. Males do not differ

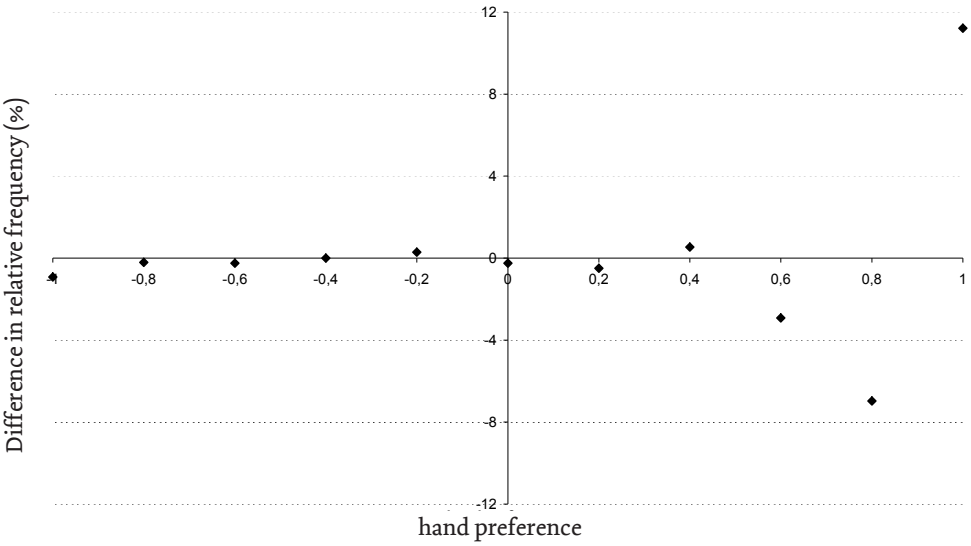

Figure 1 • Differences in the relative frequencies of the distribution of hand preference between the groups with and without schooling. Extreme right hand preference is 11.2 % more frequent and strong right preference 7% less frequent among those who went to school.

Table 4 • Effects of schooling, sex, age and age2 on task performance

Task	Factor	F(1,575)	partial eta^2	B	p-value
pegboard	schooling	17.2	0.029	-0.683	<.0001
	sex	0.085	<0.001	0.043	.770
	age	106.3	0.157	0.193	<.0001
	age2	200.8	0.26	-0.004	<.0001
gripforce		34.6	0.057		<.0001
	schooling			-3.086	
	sex	147.8	0.206	5.758	<.0001
	age	573	0.501	1.431	<.0001
	age^2	473.4	0.453	-0.02	<.0001
ball throwing		10.7	0.018		.0011
	schooling			-2.867	
	sex	14.3	0.024	2.989	.0002
	age	3.51	0.006	0.187	.061
	age^2	2.87	0.005	-0.003	.091

from females in pegboard performance. Mean skill over both hands globally decreases with age for the pegboard and increases for grip force, but these relations are non-linear with peak performance on the pegboard in the 10-29y range and on grip force in the 15-49y range.

A MANCOVA with schooling and sex as predictors and age and age^2 as covariates (df = 3,569) yielded highly significant multivariate effects (all p's < 0.00001) for all 4 factors. Univariate results are presented in Table 4. Schooling is associated with faster overall pegboard performance, greater strength and greater accuracy in throwing.

Factors predicting hand skill asymmetry

The distributions of the asymmetry of hand skill indices are shown in Figure 2. The distribution of these hand skill asymmetry indices (SI's) is close to normal with right biased means for all three tasks indicating that the majority of subjects perform better with the right than the left hand. In multivariate analyses of SI age^2 was never significant and this factor was removed from the model. The resulting multivariate model was significant for sex ($F_{3,570} = 4.93$, $p = 0.002$) and age ($F_{3,570} = 3.56$, $p = 0.014$), but not for schooling.

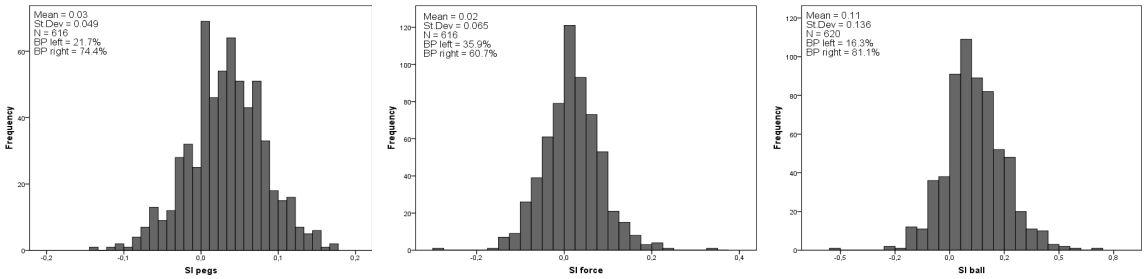

Figure 2 • Distribution of the asymmetry of hand skill (SI) for each of the three tasks. Right hand dominance in skill shows as positive values of the SI. Percentages indicate the proportion of subjects with better performance of the left hand (BP left) or the right hand (BP right).

The between-subjects effects are shown in Table 5 (top). Males had on average a higher SI (indicating that they are more right-dominant) than females in grip force ($p = 0.054$) and ball throwing ($p = 0.002$), but not on the pegboard. With age the average SI decreased for all three tasks.

The MANCOVA on absSI showed significant multivariate effects of age ($F_{3,569} = 5.16$, $p = 0.002$) and age^2 ($F_{3,569} = 5.65$, $p = 0.001$), but not of schooling. The task specific between-subjects effects are shown in Table 5 (bottom). There is a trend for stronger absolute asymmetry between the hands in males for ball throwing ($p = 0.081$), but no effects of sex on grip force and the pegboard. There is a general decrease of absSI with age for grip force and ball throwing, leveling off in the adults.

Relations between asymmetry of hand preference and hand performance

The Spearman correlations between hand preference (PI) and hand skill asymmetry (SI) for each of the three tasks are given in Table 6 (first column). Correlations concerning SIforce were not significant and only weak correlations between the other tasks were found, with less than 4% of variance explained.

Discussion

The present study addresses the influence of schooling on strength and direction of hand preference and hand skill asymmetry in a Papuan population. The main result shows that schooling is associated with hand preference and overall skill but not with hand skill asymmetry. Furthermore, we found a higher prevalence of extreme right hand preference in men compared to woman in our sample.

The validity of the hand performance measurements was evident from similar performance levels of pegboard performance compared to Western samples (e.g. Doyen *et al.* 2008), and greater grip force in men compared to women and the finding that men were better throwers than women. Also, expected quadratic effects of age on task performance show that this Papuan population is subject to the known physical developmental constraints of lower speed, force and throwing accuracy in children and the elderly compared to the adult population. Previous studies in Western populations (Steenhuis &

Table 5 • MANCOVA between-subjects effects of sex and age on the asymmetry of hand skill (left) and strength of asymmetry of hand skill (right). Schooling did not have a significant effect.

Task	Factor	F(1.575)	partial eta^2	B	p-value
pegboard	schooling	0.648	0.001	0.0037	0.421
	sex	2.48	0.004	0.0067	0.116
	age	2.91	0.005	<0.001	0.089
gripforce	schooling	0.394	0.001	-0.0039	0.53
	sex	3.72	0.006	0.0109	0.054
	age	4.99	0.009	<0.001	0.026
ball throwing	schooling	1.12	0.002	0.0135	0.29
	sex	10.1	0.017	0.0372	0.002
	age	4	0.007	-0.0007	0.046

Task	Factor	F(1.575)	partial eta^2	B	p-value
pegboard	schooling	1.99	0.003	0.0048	0.159
	sex	1.05	0.002	0.0031	0.305
	age	0.558	0.001	<0.001	0.455
	age2	0.482	0.001	<0.001	0.488
gripforce	schooling	0.087	<0.001	-0.0012	0.768
	sex	0.11	<0.001	-0.0013	0.74
	age	5.97	<0.001	-0.0012	0.015
	age2	9.82	0.017	<0.001	0.002
ball throwing	schooling	0.502	0.001	0.0073	0.479
	sex	3.06	0.005	0.0163	0.081
	age	9.48	0.016	-0.0036	0.002
	age2	7.14	0.012	<0.001	0.008

Bryden 1999; Corey *et al*. 2001; Badzakova-Trajkov *et al*. 2011) report moderate agreement between hand preference and hand skill asymmetry indices. We found low correlations between hand preference and hand skill asymmetry, and between the different skill tasks in our Papuan sample. Weak correlations between handedness measures have also been reported for a young Papuan population (Connolly & Bishop 1992). Weak correlations leave room for moderating factors such as schooling, sex, and age to have a differential effect on these measures that reflect different aspects of motor lateralization, which is indeed found in the present study.

Our results are only partially in agreement with those of Connolly & Bishop (1992) who studied a Papuan mountain population of children from Papua New Guinea. In both studies schooling was not associated with hand skill asymmetry on the Annett peg board task. However, in contrast to our study, they found no effect of schooling on observed hand preference. Other than in our study their measure of hand performance relied on performing actions with typically 'Western' objects such as dealing cards and using scissors; unfamiliarity with some of the actions may have obscured the subject's true handedness.

The effects of schooling on mean hand performance and skill asymmetry were estimated in models correcting for sex and age. A remarkable outcome of these analyses is that schooling affected mean performance but not asymmetry of hand skill or its absolute value. This may imply that hand skill is more under pressure of environmental and/or cultural influences than is skill asymmetry between the hands, but this will have to be shown for other factors than schooling. In contrast to asymmetry of hand skill, the strength and direction of hand preference was associated with schooling. Among those who had been to or still were in school extreme right-hand preference was more frequent. Together with the weak correlations between the two types of measures this suggests that hand preference and skill asymmetry represent different aspects of brain lateralization.

An unexpected finding was that the group of subjects that received at least one year of schooling performed better on each of the three tasks: schooling was associated with faster fine motor performance, greater grip strength and greater accuracy. Writing as is taught at school can be seen as a specific and intense training in fine motor control possibly explaining better performance on the skill tasks, but it is difficult to understand that this would enhance maximum grip force. An alternative explanation is that in this Papuan population, that is relatively new to the system of schooling, there is an implicit selection mechanism for school entry by parents or the broader community including the teachers, with the result that a fitter and maybe more intelligent part of the population enters school. This explanation fits with positive correlations (r = 0.18 in boys and.17

Table 6 • Spearman correlations between hand preference index (PI) and hand skill indices (SI-task) and Pearson correlations between the task SI's

	PI	SI-pegs	SI-force
SI-pegs	0.087 *	-	-
SI-force	0.032	0.045	-
SI-ball	0.188 **	0.143 **	0.040

* p<0.05 ; ** p< 0.01

in girls) between overall level of hand performance and IQ as was found in a large UK sample at the age of 11y (Nettle 2003).

Among schooled individuals we found a higher frequency in the extremely right-handed and a lower frequency in the strongly right-handed category. One might conclude that this preference is the result of pressure on writing with the right hand. Indeed there is evidence that pressure on left-handers in school to write with the right hand may convert about half of them into right-handed writers and that this also influences hand choice for a number of other tasks (see Perelle & Ehrman 1994). According to our assistants, however, pressure on writing with the right hand was not exerted. Additionally, the writing can be seen as a specific and intense training in fine motor control. For right-handers and for left-handers writing with their left hand this may cause a shift to increased strength of handedness, but for left-handers who were pressed to write with their right hand this may cause a decrease in strength of handedness. The low number of left-handed subjects in our sample (for a discussion of this finding see Schaafsma *et al*. 2011/chapter 6, this thesis) limits testing this to the full extent, but the increased frequency of extreme right-handedness and stronger strength of preference among subjects with schooling is in agreement with this reasoning. There may be, however, an alternative explanation. Going to school implies not participating in the daily routine of working in the gardens situated on steep slopes (as women and children and part of the men do) or not going up into the forests for hunting and trapping, activities that are physically demanding and require varied use of both upper limbs. Rather than an effect of writing pressure the difference in extreme right-hand preference as found between the schooled and non-schooled individuals may be caused by the more frequent bilateral use of both limbs in the living environment (school children do not work in the gardens). This hypothesis awaits further testing.

The low prevalence of left handedness in our sample (2.9%) is quite low (see also Schaafsma *et al*. 2011/chapter 6, this thesis). In many societies cultural pressure causes a bias in hand preference (Perelle & Ehrman 1994). There is as far as we were able to detect no teacher pressure to use the right hand for writing. According to our assistants, however, no cultural habits (e.g. for eating) or activities that might cause a bias were known to them. The Eipo dictionary does not report negative meanings associated with the word 'kwanim' (left) and positive meaning associated with the word 'sidik' (right) (Heeschen & Schiefenhövel, 1984), in contrast to many other languages (Schiefenhövel, in preparation). Sidik also has the meaning of preferred hand (personal observation RHG and SMS). In all, we estimate that the usual cultural pressures on hand preference have been low.

In our population we find 6% more extremely right-handed males than females. A meta-analysis of sex differences in left-hand preference by Papadatou-Pastou et al. (2008) reports an odds ratio of 1.20 for strong left-handedness (studies in which a right – mixed - left classification was used), reflecting higher prevalence of strong left-handedness among males. Although these two studies are quite different in nature and the classes of handedness only roughly similar, the common result is that among males extreme handedness is more prevalent. This may indicate that sex differences affect strength of hand preference rather than direction.

The differences in handedness between the sexes among the Papuas may be explained by the division of labour between men and women in this population. Among other tools the hunters (male) use bow and arrow, which may explain that they are better throwers but possibly also the sex difference in lateralization as they specialize the hands more than the women, who do most of the work in the gardens on steep slopes, work that is done in a position facing the mountain slope with restricted opportunity to move. This forces the use of both hands in the same task equally frequently. From these observations one may predict that among females the frequency of extreme hand preference will be reduced compared to men. This was found for extreme right hand preference (for left hand preference this study lacks power). For the pegboard task no sex differences were found, possibly because males and females are equally involved in fine motor skills. These latter results are in agreement with those of Connolly and Bishop (1992) who did not find sex differences in hand preference and pegboard performance in a Papuan population. On the other hand Doyen and colleagues (2008) reported a small but significantly stronger asymmetry for the pegboard task in females compared to males in a French sample over a similar age range (6-66y) and sample size (n = 488). And Kilshaw and Annett (1983) reported that in a large UK sample of 3.5y to 63y up to the age of 10y males are faster with their left hand, which then reverses, but no such effect was found in our data. Cultural differences or the large proportion of left-handers in the Doyen (2008) study (38%) might explain these differential effects of sex.

Conclusion

The main result of the present study on handedness in a non-industrial society is that schooling, corrected for sex and age differences, is associated with hand preference, as schooled individuals were more likely to be extremely right-handed, and less likely to be strongly right-handed, but not with hand skill asymmetry. Developmental plasticity in hand preference but not skill asymmetry and the weak correlation between hand preference and asymmetry of hand skill suggests that hand skill asymmetry is not the major determinant of hand preference and that both hand preference and hand skill asymmetry represent different aspects of brain lateralization.

The associations between schooling and hand preference are probably not merely caused by social pressures or intense training of the writing hand, but also by being less involved in activities that forces the use of both hands equally. Furthermore, the higher overall performance of schooled individuals suggests that there is implicit selection on the fitter part of the population to enter school.

Acknowledgements

We are greatly indebted to our field assistants Philipus Nabyal, Enus Kona and Yundinus Nabyal and the wonderful and helpful Eipo people. We gratefully acknowledge funding by the EU and Gratema Stichting to TGG Groothuis, the Dutch Science Foundation to TGG Groothuis and A Bouma and the Department of Psychology to RH Geuze.

References

Agresti, A. & Coull, B.A. 1998. Approximate is better than" exact" for interval estimation of binomial proportions. *American Statistician*, **52(2)**, 119–126.

Annett, M. 1992. 5 tests of hand skill. *Cortex*, **28**, 583-600.

Annett, M. 1970. A classification of hand preference by association analysis. *British journal of psychology*, **61(3)**, 303–321.

Annett, M. 1985. Left, Right, Hand and Brain: The Right Shift Theory. Erlbaum., Hillsdale, NJ.

Bishop, D. 1990. Handedness and developmental disorder, London: Mac Keith.

Borod, J., Caron, H. & Koff, E. 1984. Left-handers and right-handers compared on performance and preference measures of lateral dominance. *British journal of Psychology*, **75**, 177-186.

Bradshaw, J. & Rogers, L. 1996. Tool use and evolutionary development of manual asymmetry. In D. Elliott & EA Roy, eds. *Manual asymmetries in motor performance*. New York: CRC Press, pp. 33-54.

Bryden, M., Ardila, A. & Ardila, O. 1993. Handedness in native amazonians. *Neuropsychologia*, **31(3)**, 301-308.

Cavill, S. & Bryden, P. 2003. Development of handedness: Comparison of questionnaire and performance-based measures of preference. *Brain and Cognition*, **53(2)**, 149-151.

Connolly, K.J. & Bishop, D.V. 1992. The measurement of handedness: a cross-cultural comparison of samples from England and Papua New Guinea. *Neuropsychologia*, **30(1)**, 13-26.

Corey, D.M., Hurley, M.M. & Foundas, A.L. 2001. Right and left handedness defined: A multivariate approach using hand preference and hand performance measures. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology*, **14(3)**, 144-152.

Doyen, A.-L., Dufour, T., Caroff, X., Cherfouh, A. & Carlier, M. 2008. Hand preference and hand performance: cross-sectional developmental trends and family resemblance in degree of laterality. *Laterality*, **13(2)**, 179-97.

Faurie, C. & Raymond, M. 2005. Handedness, homicide and negative frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, **272(1558)**, 25-28

Faurie, C., Schiefenhövel, W., Le Bomin, S., Billiard, S. & Raymond, M. 2005. Variation in the Frequency of Left- handedness in Traditional Societies. *Current Anthropology*, **46**, 142-147.

Gilbert, A.N. & Wysocki, C.J. 1992. Hand preference and age in the United States. *Neuropsychologia*, **30(7)**, 601–608.

Holder, M. & Kateeba, D. 2004. Hand preference survey of 5136 school children in Western Uganda. *Laterality*, **9(2)**, 201–207.

Kilshaw, D. & Annett, M. 1983. Right-and left-hand skill: I. Effects of age, sex and hand preference showing superior skill in left-handers. *British Journal of Psychology*, **74 (Pt 2)**, 253-268.

Marchant, L.F. & McGrew, W.C. 1998. Human handedness: an ethological perspective. *Human Evolution*, **13(3-4)**, 221-228.

Medland, S., Perelle, I., De Monte, V. & Ehrman, L. 2004. Effects of culture, sex, and age on the distribution of handedness: An evaluation of the sensitivity of three measures of handedness. *Laterality* **9(3)**, 287-297.

Nettle, D., 2003. Hand laterality and cognitive ability: A multiple regression approach. *Brain and Cognition*, **52(3)**, 390-398.

Papadatou-Pastou, M., Martin, M., Munafò, M.R. & Jones, G.V. 2008. Sex differences in left-handedness: a meta-analysis of 144 studies. *Psychological bulletin*, **134(5)**, 677-99.

Perelle, I.B. & Ehrman, L. 1994. An international study of human handedness: the data. *Behavior Genetics*, **24(3)**, 217-27.

Ploeg, A. 2004. The German Eipo Research Project. *Journal de la Société des Océanistes*, **118**, 35-79.

Raymond, M., Pontier, D., Dufour, A.B. & Møller A.P. 1996. Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society: Biological Sciences*, **263(1377)**, 1627–1633.

Roy, E, Bryden, P. & Cavill, S. 2003. Hand differences in pegboard performance through development. *Brain and Cognition*, **53(2)**, 315-317.

Schaafsma, S., Geuze, R.H., Riedstra, B., Schiefenhövel, W., Bouma, A. & Groothuis, T. Handedness in a non-industrial society challenges the fighting hypothesis as an evolutionary explanation of left-handedness. *Evolution & Human Behavior*, In press.

Schiefenhövel, W. 1997. Reactions to cultural change. Among the Eipo in the highlands of West-New Guinea. *Civilisations*, **44**, 220-227.

Steenhuis, R.E. 1999. The relation between hand preference and hand performance: what you get depends on what you measure. *Laterality*, **4(1)**, 3-26.

Triggs, W.J., Calvanio, R., Levine, M., Heaton, R.K. & Heilman, K.M. 2000. Predicting hand preference with performance on motor tasks. *Cortex*, **36(5)**, 679-89.

Volkmann, J. , Schnitzler, A., Witte, O.W., Freund, H.-J. 1998. Handedness and asymmetry of hand representation in human motor cortex. *Journal of Neurophysiology*, **79(4)**, 2149-2154.


PARACETAMOL
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Part II

Ultimate factors underlying lateralization

6

Handedness in a nonindustrial society challenges the fighting hypothesis as an evolutionary explanation for left-handedness

Sara M Schaafsma, Reint H Geuze, Bernd Riedstra, Wulf Schiefenhövel, Anke Bouma & Ton G G Groothuis • Evolution and Human Behavior, in press.

Abstract

Handedness is a heritable trait, and left-handedness is related with increased fitness costs. Left-handedness persists, however, as a minority in every human population investigated. One explanation for this persistence has been put forward in the fighting hypothesis, which postulates that left-handers have a frequency dependent benefit in fights. Support for this has been found in the finding that left-handedness is relatively frequent in populations with high homicide rates, according to estimates of left-handedness partly based on pictures and films made for a different purpose. We measured handedness based on nonindustrial society of the Eipo (Papua, Indonesia) in which homicide rate was very high. This set of tests was validated in 198 Western students. Contrary to the prediction based on the fighting hypothesis, we did not find a high frequency of left-handedness or a difference between men (who participate in warfare) and women evolutionary force for the persistence of left-handedness in human populations. Furthermore, we found lower percentages of left- and mixed-handers compared to a Western population who executed the same tasks. Since left-handedness is associated with health problems, we suggest that in a society lacking Western health care, selection pressures against left-handedness may be more intense and therefore its frequency may be reduced.

Introduction

Left-handed people are present in every human population investigated but always comprise a small minority compared to the large majority of the population that is right-handed. Handedness is a heritable trait (Sicotte *et al.* 1999; Francks *et al.*, 2002; Medland *et al.* 2009), and left-handedness is correlated with increased Darwinian fitness costs (see references in Llaurens *et al.* 2009). One way to explain the persistence of the minority of left-handers in human societies is by negative frequency dependent selection: pressure against a certain phenotype (in this case, left-handedness) decreases in the population when it becomes less frequent relative to the other phenotype (right-handedness) since its benefits are higher when rare. The fighting hypothesis (Raymond *et al.* 1996) postulates that such a frequency-dependent benefit for left-handers could be found in an advantage they have in fights because of their unfamiliar handedness. People engaged in fights have a higher chance of encountering right-handed opponents and are therefore better prepared to fight against right-handers, thus giving left-handers a better chance of winning fights as long as left-handedness is rare. This hypothesis is supported by the finding that in physically interactive sports (e.g. fencing and tennis), the frequency of left-handers is relatively high compared to noninteractive sports (e.g. gymnastics) (Raymond *et al.* 1996). A further prediction resulting from this hypothesis is that when fighting is more frequent in a society, the frequency-dependent benefit of left-handers becomes larger, either due to the increased survival in fights or due to indirect effects of winning a fight by an increase in social rank, possibly resulting in more sexual partners and thereby more offspring. Indeed, in a comparative study of eight nonindustrial societies, it was found that the percentage of left-handers (varying between 3.4% and 22.6%) is positively correlated with the number of adult homicides per year (Faurie & Raymond 2005). Faurie & Raymond (2005) reported handedness data from three populations with high homicide rates. In two of these populations, handedness was estimated based on photos and film recordings that were made for a different purpose: in the Yanomamö (Brazil), lateralized behaviors were scored from 16-mm film recordings of daily life of a small number of people (n=31, Marchant *et al.* 1995); and in the Eipo (Papua, Indonesia), pierced ears were scored from pictures taken during prior fieldwork. A pierced left ear would indicate that bow shooting is performed with the right hand and vice versa as bringing the bow under tension by pulling the string to the side of the face would be hampered by large ornaments in the ear on that side of the face. These data were subsequently corrected for the chance that the unseen earlobe on pictures was torn in the process of enlarging the hole, in which case these men would often also pierce their other ear (ipsilateral to bow use) (Faurie *et al.* 2005). Since other societies in the comparative study by Faurie & Raymond (2005) were scored on the basis of hand use while using a knife, they adjusted the Eipo data based on the between handedness in bow shooting and knife use in a small French population. In the population reported to have the highest homicide rate (Jimi Valley, Papua New Guinea), handedness measures were obtained through testing (Connolly & Bishop, showed a frequency of left-handedness that was considerably lower than in the other two studies.

The fighting hypothesis is at present the only evolutionary explanation for the persistence of left-handedness in human populations, and the result of the comparative approach by Faurie & Raymond (2005) is intriguing. To further investigate whether the proportion of left-handedness is high in populations with high levels of homicide, we investigated handedness in the Eipo population on the basis of actual preference in standardized tests. In this nonindustrial society (belonging to the Mek group of cultures and languages), both homicide rate is high (3/1000 inhabitants per year; Schiefenhövel, 2001), and Faurie & Raymond (2005) estimated the rate of left-handedness to be very high too (20.4%). Furthermore, in addition to differentiating between right-and left-handers, we also differentiated between right-and mixed-handers since the latter may profit from the same advantage in fights as left-handers. To measure handedness in an evolutionarily meaningful manner, we developed a series of preference tests in which handedness was scored on 10 actions common to subjects' daily lives. This 10-item inventory was applied in the Papuan population, and the data were compared with data from a Dutch (student) population in which the same 10-item inventory was completed in a similar way. To investigate whether the student sample was representative of the Western population, the student results were compared to data collected by Annett (2004) who measured handedness in a Western population with a series of tests based on tasks common to Western daily life.

Since the Eipo accepted Christianity as their primary religion around 1980 (Ploeg 2004), tribal wars came to an end, and thus homicide rate diminished dramatically. There has only been one reported fatal outcome of an intracommunity fight since then (personal communication, W. Schiefenhövel). Consequently, since the acceptance of Christianity, the benefit of being left-handed may have been reduced. Therefore, we tested whether handedness differed between people who were adults in the period when tribal wars were common and those too young to take part in these or not yet born. As only the men participate in the fights and women would benefit from being left-handed only indirectly (as left-handedness is heritable) via their left-handed sons, this analysis was repeated for men only, and it was analyzed whether handedness differed between the sexes.

Methods

The Papuan sample

The Eipo inhabit an area of around 150 km2 along the banks of the Eipomek river at approximately 4°25`–4°27` S, 140°00`–140°05` E in the highlands of the Indonesian province of Papua, formerly known as Irian Jaya (New Guinea). The inhabitants are horticulturists whose staple food consists of sweet potatoes and vegetables, complemented by the products of hunting, gathering and pig raising (Schiefenhövel 1976, 1991; personal observations 2009). Because of the remoteness and inaccessibility of the area the Eipo valley is located in, it has until recently been isolated from the outside world. The first brief contact with Europeans was in 1959 during the expedition led by Gaisseau (Saulnier 1960). Tribal warfare and armed conflict within the village communities were common until missionaries began their work in the valley in 1978, and in 1980, Christianity was accepted as the primary religion (Ploeg 2004). Western health care was absent until 2005

when a health center was built that now offers basic health care. In the valley, there has been one elementary school since 1981. No fees are required to attend this school; however, many children do not attend school for various reasons (e.g. to be able to help their parents in the gardens). At present, the area is accessible by foot or light aircraft only.

Because adults do not know their own age or the age of their children, the interviewers estimated the age of each subject based on a series of major events in the community of which exact dates were known from documented record (such as the first contacts with Europeans, Indonesian parachutists in 1969, the arrival of the German Research Team in 1974, two destructive earthquakes in 1976 and the construction of the school and church) that the subjects recollected. Our estimates of the age of the subjects ranged between 5 and 69 years of age (median = 27, interquartile range = 31). All observations were done by the first two authors with the help of three local Eipo assistants who translated the instructions from Indonesian to the local language (*Eipo yupe*). In all cases, one observer and one interpreter were present when testing a subject. The interpreters were blind to the hypotheses being tested. The project was introduced to the local people by W. Schiefenhövel, who speaks Eipo (cp. Heeschen & Schiefenhövel 1983) and is known to them from repeated periods of field work that started in 1974 when the Eipo still lived their traditional Neolithic life style.

Handedness measures

Hand preference was determined in 621 Eipo subjects (289 men and 332 women) using tools provided for demonstration. Subjects gave verbal informed consent prior to the assessment, which was approved by the ethics committee of the University of Groningen. The inventory consisted of 10 actions the subjects performed and comprised both fine and gross motor skills, namely, (1) one punch at a bag held up by an observer (mimicking giving someone a punch during a fight); (2) sharpening a wooden stick with a knife (hand used to handle knife was recorded); (3) hammering a wooden stick into the ground with a stone (hand used to handle stone was recorded); (4) machete use (cutting vegetation); (5) throwing a small stone far away; (6) picking up a nut and putting it in the mouth; (7) picking up a bead and handing it over to the observer; (8) drawing a circle on the ground with a wooden stick; (9) swatting away an imaginary fly located on the subject's nose and (10) crushing a small stone with a big stone. The tasks comprised of both unimanual (Nos. 1, 4, 5, 6, 7, 8, 9 and 10) and bimanual items (Nos. 2 and 3). The tools were bimanually placed precisely in front of the subject in such a way that no cue for left- or right-hand use was given by a bias in the placement of tools. A score of 1 was given to each action performed with the right hand and –1 to an action performed with the left hand. A handedness index (HI) was calculated by dividing the total score by 10. The HI thus ranged between –1 and 1. For unimanual tasks, it was explicitly asked to use one hand only. If a subject then used both hands, the data from this subject were excluded from statistical analyses (n = 19).

The Western sample and validation of the tests

Data were collected during first year biology practicals in the same way as described above for the Eipo population. All subjects (102 men and 85 women; median = 19 years old, interquartile range = 1 year) gave written informed consent prior to testing, which was approved by the ethics committee of the University of Groningen. Following the same criteria as for the Eipo population, 10 students were excluded as hand use could not be properly scored on all 10 items.

To investigate whether the HI obtained with the tasks (developed for a Papuan society) was comparable to the HI obtained with questionnaires generally used in Western populations, the students also completed the Dutch handedness questionnaire (van Strien 2003). This questionnaire is based on the handedness questionnaires of Annett (1970) and the Edinburgh inventory (Oldfield, 1971). Additionally, to investigate whether the HI obtained from the student population by means of testing actions designed for the Papuan population was similar to results obtained from other Western populations, the data were compared to data collected by Annett (2004) in a large Western sample (UK) with a broader age range using action tasks designed for a Western society. Both approaches showed that the test series on hand preference developed for the Papuan population in the students produced similar data as the questionnaire did, and the test series on hand preference developed for Western populations collected by Annett (2004) verified that the test series we designed for the Papuan population indicates handedness reliably in our student population. For more detailed information concerning these comparisons, please refer to the Supplementary material.

Analyses

Comparing Faurie & Raymond's (2005) with our Papuan sample

First, we compared our data to the data in the Eipo population reported by Faurie & Raymond (2005) who calculated that 20.4% of men in that population were left-handed (aged over approximately 15 years as ears were pierced only after the start of puberty). This calculation was solely based on an estimation of handedness in knife use (see Introduction). To make a proper comparison, we only used the knife test from our data set and restricted our sample to men older than 15 years. We performed a binomial test (Siegel 1956) on the number of left-handers with 20.4% as reference. Additionally, as our HI based on all 10 tests resulted in an ordinal handedness measure between –1 and 1 (in contrast to the nominal left or right hand use in Faurie & Raymond, 2005), we also performed a binomial test comparing the 20.4% left-handers estimated by Faurie & Raymond with the percentage of people having an HI smaller than zero.

In addition, among the Papuans, we tested whether older men, having experienced warfare, differed from younger people and whether men differed from women who do not participate in warfare. For details, see the Supplementary material.

Comparing Papuan and Western distributions of hand preference

Due to the narrow age range of our student sample, we selected a subsample of our Papuan sample ($16 < age < 26$) when the Papuan population was compared with the student population (after selection: median = 20, n = 71 and median = 19, n = 187, respectively).

A Kolmogorov–Smirnov (KS) Exact Test was used to analyze the differences between the two populations. Because we found low frequencies of left-handedness in the Papuan population, we used a conservative measure to test more specifically the differences in left-and mixed-handedness by means of χ^2 tests of independence (Siegel 1956) with the criteria of HI<0 for left-handedness and $-0.7 < HI < 0.7$ for mixed-handers. After visually inspecting the data, we also analyzed the differences between the populations for extreme right-handedness (HI>0.9).

Statistical programs

Binomial tests and χ^2 tests for independence were performed using the software program Statistix version 8 (Analytical Software, Tallahassee, FL, USA, 2003). All other statistical analyses were performed using SPSS 16.

Results

The overall results concerning the HI of Papuan individuals are shown in Table 1.

Comparing Faurie & Raymond's (2005) with our Papuan sample

Using HI<0 as a cutoff point to determine left-handedness in the male population older than 15 years, we found seven left-handers out of a total of 194 Papuan individuals (3.6%). This percentage significantly differed from 20.4% reported by Faurie & Raymond (2005) (binomial test, $p < 0.001$). Using only the results of the single item a wooden stick with a knife' (equal to the left-handedness measure estimated by Faurie & Raymond, 2005) yielded exactly the same results. Further analyses showed that men were not more likely to be left-handed than were women. Similarly, Papuans who had experienced warfare were not more likely to be left-handed than were younger people (see supplementary material pp. 115-117).

Comparing nonindustrial with Western left-handedness

The Papuan HI distribution (mean ± SD = 0.98 ± 0.074, N = 71) was significantly shifted to the right of the Dutch sample (mean ± SD = 0.81 ± 0.461, N = 187; KS Exact Test: $Z = 1.51$, $p < 0.001$) (see figure 1). Numbers of individuals and percentages of strong left-, left-, mixed-, strong right- and extreme right-handers are shown in Table 2. The percentage of the left-and mixed-handers was significantly lower in the Papuan sample than in our Western student sample ($\chi^2 = 4.36$, $p = 0.037$ and $\chi^2 = 4.49$, $p = 0.034$, respectively). The percentage of strong and extreme right-handers was significantly higher in the Papuan sample than in the Western student sample ($\chi^2 = 7.84$, $p = 0.005$ and $\chi^2 = 13.85$, $p = 0.0002$, respectively).

Table 1 • Frequency distribution (number and percentage) of handedness in the Papua population.

Handedness Index	-1.0	-0.8	-0.6	-0.4	-0.2	0	0.2	0.4	0.6	0.8	1.0
N (%)	10 (1.7)	4 (0.7)	1 (0.2)	1 (0.2)	2 (0.3)	1 (0.2)	2 (0.3)	1 (0.2)	13 (2.2)	41 (6.8)	526 (87.4)

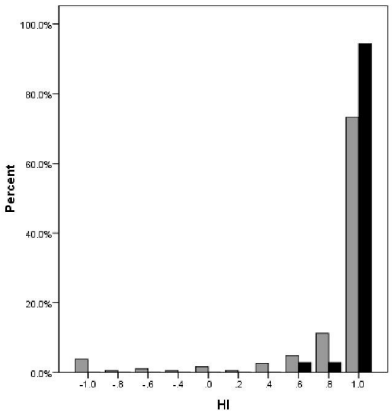


Figure 1 • Frequency distribution of the HI of Papuan (black bars) and Western (gray bars) subjects between 16 and 26 years old. Papuan subjects were significantly less often left-handed ($\chi^2 = 4.36$, $p = .037$) and mixed-handed ($\chi^2 = 4.49$, $p = .034$) and more often right-handed ($\chi^2 = 7.84$, $p = .005$) than Western subjects.

Discussion

In the present study, we did not find elevated frequency of left-handedness in a non-industrialsociety with high homicide rate as was expected on the fighting hypothesis postulated by Raymond *et al.* (1996).The percentage of left-handedness we found was substantially and significantly lower than the percentage reported by Faurie & Raymond (2005) and also lower than what we found in a Western (student) population using the same tests. As no difference in the proportion of left-handedness was evidence that the dramatic cultural change following the conversion to Christianity that nearly eliminated homicide had any effect on the percentages of left-handedness. These results thus challenge the fighting hypothesis. We also investigated differences in the proportion of lefthanders between the sexes, as men but not women participated in warfare, so that the latter may only receive benefits from being left-handed through their sons. In the literature, a small sex difference in handedness has often been found, with men showing a slightly higher frequency of left-handedness than women (for a meta-analysis, see Papadatou-Pastou *et al.* 2008). Although Papuan men were more often left-handed than women, this difference was not significant, perhaps due to an insufficient sample size.

Table 2 • Number of individuals and percentages of strong left -, left -, mixed-, strong right- and extreme right-handedness in the nonindustrial and Western populati on and the associated χ2 tests of independence

	Strong left-handers HI<-0.7		**Left-handers** HI<0		**Mixed-handers** -0.7<HI<0.7		**Strong right-handers** HI>0.7		**Extreme right-handers** HI>0.9	
	N	%	N	%	N	%	N	%	N	%
Non-industrial sample	0	0	0	0	2	2.8	69	97.2	67	94.4
Western sample	8	4.2	11	5.9	21	11.2	158	84.5	137	73.3
$\div^2$	3.13		4.36		4.49		7.84		13.85	
p	0.077		0.037		0.034		0.005		0.0002	

Subject age: between 16 and 26 years.

Although our newly collected data on handedness are not consistent with the fighting hypothesis, considering the way homicide occurs in this population, the hypothesis may still be valid. In the populations that are recorded to have the highest homicide rate (Faurie & Raymond 2005), people mainly fight with bow and arrow (Yanomamö: Chagnon 1988; Jimi valley: Vayda 1989; Eipo: Schiefenhövel 2001). In the absence of hand-to-hand combat, there is no reason to expect any frequency-dependent benefit for left-handers because it should not matter whether an arrow is aimed or released with the left or the right hand. Therefore, instead of correlating homicide rate to percentages of left-handers in human populations, the number of man-to-man fights should be used as this is a more accurate measure of the possible frequency-dependent benefit of left-handers. However, even if such a correlation would be found and left-handers do indeed have higher chances to win such fights, it is difficult to disentangle whether this would be caused by frequency-dependent advantage or by better motor skills, higher aggression among left-handers or other differences in lateralized functions such as spatial explanations that can also be invoked to relatively high percentage of left-handers sports compared to noninteractive sports (Bisiacchi *et al.* 2005; Reio 2004; discussed in Harris 2010).

Contrary to expectation, left-handedness was relatively low in the Papuan society even when compared to a Western population. An alternative for the fighting hypothesis is based on the relationship between left-handedness and may be a side effect of developmental disorders or perinatal stress factors that selection cannot bring to extinction. Several authors have suggested that left-handedness (see references in Llaurens *et al.* 2009), non-right-handedness (see references in van der Hoorn *et al.* 2010) or being weakly lateralized (Vallortigara 2006) is related to fitness costs such as decreased health. The decreased levels of both left-handedness and mixed-handedness in the Papuan sample compared to a Western population may be due to the formerly absent and still very limited Western health care in this Papuan population (Braun 1996; Schiefenhövel 1976), leading to higher mortality in the Eipo than in typically studied Western populations with their sophisticated health care system.

In addition to a low proportion of left-handedness, we found a higher proportion of extreme right-handedness at the expense of less extreme right-handedness compared to a Western population. This result is in agreement with Connolly and Bishop (1992) who compared handedness in similar nonindustrial society (Jimi Valley, Papua New Guinea) and a Western population (Manchester, UK). They attributed their result to the fact that they investigated handedness with an inventory based on the daily lives of Western people and that the tasks were not completely appropriate for testing handedness of the people in the Jimi valley. However, we find the same results even though the tasks were developed for daily nonindustrial life of that specific society.

Connolly and Bishop (1992) also report that, during the performance of the tasks, there always was a crowd of people watching, a situation similar to ours. Following their reasoning, it is possible that the Papuans may have been more preoccupied with the idea that they should perform the task in a correct and timely manner, whereas the students may just have used the hand they normally use when performing a certain task. This might have caused the shift of hand preference to more extreme right-handedness.

In conclusion, our study contradicts the estimation of extremely high frequency of left-handedness in the Eipo society by Faurie & Raymond (2005). This weakens the support for the fighting hypothesis and is not in line with the general predictions of this hypothesis. Moreover, we found lower instead of higher levels of left-and mixed-handedness in this society when compared to a Western population with much lower incidence of homicide. Since left-handedness is related to increased fitness costs, our finding may be explained by the lack of Western health care, resulting in increased selection pressures against left-handedness in the nonindustrial society investigated. Possibly, investigating the relationship between levels of health care and left-handedness between populations may be a next step to shed light on the variation and persistence of left-handedness in human populations.

Acknowledgements

We gratefully acknowledge funding by the EU to TGG Groothuis, the Dutch Science Foundation to TGG Groothuis and A Bouma, the Gratema Stichting to TGG Groothuis and the Department of Psychology to RH Geuze. We are greatly indebted to our field assistants, Philipus Nabyal, Enus Kona, Yundinus Nabyal and Alex Verkade, and the kind and helpful Eipo people. We thank Jessica Lust for her contribution in the preparation of this study.

Supplementary material

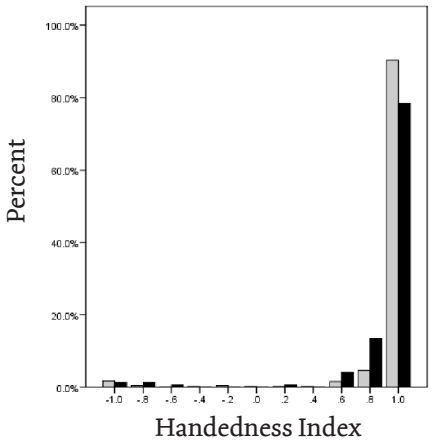
The Western sample and validation of the tests

To investigate whether the handedness index (HI) obtained from the student population (102 men and 85 women; median = 19 years old, inter-quartile range = 1 year) was similar to other Western populations, the data was compared to data collected by Annett (2004) in a large UK sample with a broader age range and action tasks designed for a western society. This sample consisted of 785 secondary school children aged approximately between 11 and 17 years and 1603 university participants aged between 18 and 63 years. Annett found that 6 out of 12 items were highly correlated and she analysed these separately in addition to analyses concerning all 12 tasks. In order to compare our data to the study of Annett we reduced the number of tasks from the 10-item inventory to a 6-item inventory by selecting the items that showed highest internal consistency. Internal consistency was calculated as the coefficient of the correlation between a specific item and the handedness index of the other 9 items. The six items with highest internal consistency comprised throwing a small stone far away (r = 0.888), punching a bag (r = 0.841), machete use (r = 0.819), crushing a small stone with a big stone (r = 0.812), hammering a wooden stick into the ground with a stone (r = 0.780) and sharpening a wooden stick with a knife (r = 0.778). The other r's varied between 0.760 and 0.566. A new handedness index was calculated based on these 6 items and categorized into three groups which were also used by Annett: extreme right-handedness (HI = 1), mixed handedness (1 < HI < -1) and extreme left-handedness (HI = -1). These results were compared with Annett's data (2004) by means of chi-square tests of independence. The percentages of left, mixed, and right-handers based on this new handedness index were not significantly different from the percentages of left, mixed, and right-handers found by Annett (Supplementary table 1). This suggests that the student population is a representative sample of the Western population.

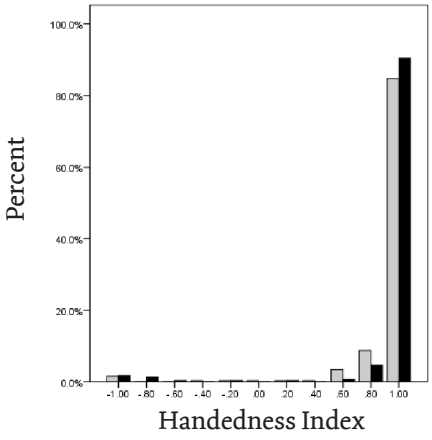
Furthermore, a Spearman's correlation between the handedness index obtained by means of the inventory of 10 tasks developed for the Papuan sample and the handedness index obtained through the standardized questionnaire with normative data of the Dutch population was performed. A strong correlation was detected between the handedness index obtained by means of the inventory of 10 actions developed for the Papuan sample and the handedness index obtained through the questionnaire (Pearson r = 0.94, N = 187, p<0.000001).

Supplementary table 1 • The percentages of hand preference categories in our Western student sample and in Annett 's (2004) sample and the associated chi-square tests of independence

	Consistent left-handers	**Mixed-handers**	**Consistent right-handers**
Students (this study)	4.3	14.9	80.7
Annet (2004)	5.2	17.3	77.6
χ^2	0.30	0.66	1.00
p	0.585	0.4171	0.3177



Supplementary figure 1 • Frequency distribution of the handedness index of Papuan people that were aged ≥15 years in 1980 (black bars) and younger ones (grey bars). No differences were found between the percentages of left- and mixed-handers between the younger and older generation. The younger generation is more oft en extreme right-handed compared to the older generation (χ^2 = 14.40, p=0.0001).



Supplementary figure 2 • Frequency distribution in percentages of the handedness index of 602 Papuan subjects. Men: black bars, women: grey bars. No differences were found between the percentages of left - and mixed-handers between the sexes. However, men are more often extreme right-handed than women (χ^2 =4.35, p=0.037).

Handedness indices of the young and old Papuan population

In 1980 instances of armed inter and intra-group aggression reduced very much due to the activity of Christian missionaries. Consequently, the benefit of being left-handed may have been reduced. To investigate whether the handedness distribution changed since that time we split the data set in two: one group who had been 15 years or older in 1980 (people who may have been involved in armed conflict) and one group who was 14 years or younger in 1980. A Kolmogorov-Smirnov Exact test was used to check for differences in the distribution of handedness between these two age classes. Additionally, chi-square tests of independence were used to investigate more specifically the differences in the proportion of left-handedness (HI < 0) in the two groups. These two analyses were done for both sexes together and separately for men as only men would have a direct frequency-dependent advantage of being left-handed.

These analyses showed that the handedness index distribution of people that were aged ≥ 15 years in 1980 when the wars were abandoned was significantly different from the handedness index distribution of people from the younger group (mean ± SD = 0.89 ± 0.346, n = 148 and mean ± SD = 0.93 ± 0.315, n = 454 respectively; KS Exact test Z = 1.260, p < 0.001; Supplementary figure 1). The percentages of strong left-, left-, mixed-, strong right- and extreme right-handers are shown in Supplementary table 2. Chi-square tests of independence showed that this difference was not caused by differences in the proportion

Supplementary table 2 • Number of Papuan individuals and percentages of strong left-, left-, mixed-, strong right- and extreme right-handers born after 1965 and born in 1965 or earlier and the associated chi-square tests of independence for both sexes together and men only.

		Strong left-handers HI<-0.7		Left-handers HI<0		Mixed-handers -0.7<HI<0.7		Strong right-handers HI>0.7		Extreme right-handers HI>0.9	
		N	%	N	%	N	%	N	%	N	%
Total	**>1965**	10	2.2	13	2.9	13	2.9	431	94.9	410	90.3
	=1965	4	2.7	5	3.4	8	5.4	136	91.9	116	78.4
	χ^2	0.12		0.1		2.14		1.89		14.4	
	p	0.726		0.749		0.143		0.17		0.0001	
Men only	**>1965**	6	3.2	7	3.7	2	1.1	182	95.8	177	93.2
	=1965	3	3.3	4	4.4	3	3.3	85	93.4	77	84.6
	χ^2	0		0.08		1.77		0.74		5.17	
	p	0.951		0.774		0.183		0.39		0.023	

of left-handers, nor in the proportion of mixed-handers. Rather, the difference lay in the fact that the younger generation was more extreme right-handed (Supplementary Table 2).

When only men were considered (the sex directly involved in fights) we also found that the HI distribution of people that were aged ≥ 15 years in 1980 (mean ± SD = 0.890 ± 0.381, n = 91) was significantly different from the HI distribution of younger group (mean ± SD = 0.925 ± 0.351, n = 190; KS Exact test Z = 0.67, p = 0.031). Chi-square tests of independence showed that this difference was again not caused by differences in the proportion left-handers, or in the proportion mixed-handers. Rather, the younger generation was more extreme right-handed (Supplementary Table 2).

Investigating differences in handedness index between the sexes

A Kolmogorov-Smirnov Exact test was used to check for differences in the distribution of handedness between men and women in the Eipo society. This analysis showed that the handedness index distribution of men (mean ± SD = 0.914 ± 0.36, n = 281) significantly differed from that of women (mean ± SD = 0.922 ± 0.287, n = 321) (KS Exact test Z=0.69, p=0.05; Supplementary figure 2).

Additionally, chi-square tests of independence were used to investigate differences in the proportion of left- and mixed-handers (HI<0 and -0.7<HI<0.7, respectively) between men and women. This showed that the sex difference found was not caused by differences in the proportion left-handers (2.2% women and 3.9% men used predominantly their left-hand to perform the 10 tasks: $\chi^2 = 1.55$, p = 0.213), or in the proportion of non-right-handers (6.5% women and 5.0% men: $\chi^2 = 0.67$, p = 0.415), but the difference lay in the fact that men are more extreme right-handed (84.7% women and 90.4% men used their right-hand on all 10 items: $\chi^2 = 4.35$, p = 0.037).

References

Annett, M. 1970. A classification of hand preference by association analysis. *British Journal of Psychology*, **61**, 303 321.

Annett, M. 2004. Hand preference observed in large healthy samples: classification, norms and interpretations of increased non-right-handedness by the right shift theory. *British Journal of Psychology*, **95**, 339 353.

Bisiacchi, P.S., Ripoll, H., Stein, J.F., Simonet, P., & Azemar, G. 1985. *Perceptual and Motor Skills*, **100(3)**, 743 746.

Braun, A. 1985. Häufigkeit und Verteilung von Erkrankungen bei zwei Naturvölkern Neuguineas. Berlin: Verlag für Wissenschaft und Bildung.

Chagnon, N. 1988. Life histories, blood revenge, and warfare in a tribal population. *Science*, **239**, 985–992.

Connolly, K. J., & Bishop, D. V. 1992. The measurement of handedness: a cross-cultural comparison of samples from England and Papua New Guinea. *Neuropsychologia*, **30**, 13–26.

Dane, S., & Şekertekin, M. A. 2005. Differences in handedness and scores of aggressiveness and interpersonal relations in soccer players. *Perceptual and Motor Skills*, **100(3)**, 743–746.

Faurie, C., & Raymond, M. 2005. Handedness, homicide and negative frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 25-28.

Faurie, C., Schiefenhövel, W., le Bomin, S., Billiard, S., & Raymond, M. 2005. Variation in the frequency of left-handedness in traditional societies. *Current Anthropology*, **46**, 142 147.

Francks, C., Fisher, S., MacPhie, I., Richardson, A., Marlow, A., Stein, J., & Monaco, A. 2002. A genomewide linkage screen for relative hand skill in sibling pairs. *American Journal of Human Genetics*, **70**, 800 805.

Harris, L. J. 2010. In fencing, what gives left-handers the edge? Views from the present and the distant past. *Laterality*, **15**,15 55.

Heeschen, V., & Schiefenhövel, W. 1983. Wörterbuch der Eipo-Sprache. Eipo-Deutsch-English. Berlin: D. Reimer

Heeschen, V., Schiefenhövel, W., Eibl-Eibesfeldt, I. 1980. Requesting, giving, and taking: the relationship between verbal and nonverbal communication in the speech community of the Eipo, Irian Jaya (West New Guinee). In M.R. Key, ed. *The relationship of verbal and nonverbal communication*. The Hague, The Netherlands: Mouton Publishers. pp. 139-166

Llaurens, V., Raymond, M., & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical transactions of the Royal Society of London. Series B. Biological Sciences*, **364**, 881 894.

Marchant, L. F., McGrew, W. C., & Eibl-Eibesfeldt, I. 1995. Is human handedness universal? from three traditional cultures. *Ethology*, **101**, 239 258.

Medland, S. E., *et al*. 2009. Genetic influences on handedness: data from 25,732 Australian and Dutch twin families. *Neuropsychologia*, **47(2)** 330-337

Oldfield, R. C. 1971. The assessment and analyses of handedness: the Edinburgh inventory. *Neuropsychologia*, **9**, 97 113.

Papadatou-Pastou, M., Martin, M., Munafo, M. R., & Jones, G. V. 2008. Sex differences in left-handedness: a meta-analysis of 144 studies. *Psychological Bulletin*, **134**, 677 699.

Ploeg, A. 2004. The German Eipo Research Project. *Journal de la Société des Océanistes*, **118**, 35 79.

Raymond, M., Pontier, D., & Dufour, A., Møller A.P. 1996. Frequency-dependent maintenance of left-handedness in humans. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 1627 1633.

Reio, T. G., Czarnolewski, M., & Eliot, J. 2004. Handedness and spatial ability: differential patterns of relationships. *Laterality*, **9**, 339 358.

Saulnier, T. 1960. Les Papous coupeurs de têtes: 167 jours dans la préhistoire. Editions du Pont Royal: Paris.

Schiefenhövel, W. 1976. Die Eipo-Leute des Berglands von Indonesisch-Neuguinea: Kurzer Überblick über den Lebensraum und seine Menschen. Einführung zu den Eipo-Filmen des Humanethologischen Filmarchivs der Max-Planck-Gesellschaft. *Homo*, **26(4)**, 263 275.

Schiefenhövel, W. 1991. Eipo. In T. E. Hays, ed. *Encyclopedia of world cultures, Vol. II, Oceania*. Boston: G.K.Hall & Co. pp. 55-59

Schiefenhövel, W. 2001. Kampf, Krieg und Versöhnung bei den Eipo im Bergland von West-Neuguinea-Zur Evolutionsbiologie und Kulturanthropologie aggressiven Verhaltens. In W. Fikentscher, ed. (Hrsg.) *Begegnung und Konflikt-eine kulturanthropologische Bestandsaufnahme. Bayerische Akademie der Wissenschaften, Philosophisch-Historische Klasse, Abhandlungen, Neue Folge, Heft 120.* München: C.H. Beck. pp. 169-186

Siegel, S. 1956. Nonparametric statistics for the behavioural sciences. New York: McGraw-Hill.

Sicotte, N., Woods, R., & Mazziotta, J. 1999. Handedness in twins: a meta-analysis. *Laterality*, **4**, 265 286.

Vallortigara, G. 2006. The evolutionary psychology of left and right: costs and benefits of lateralization. *Developmental Psychobiology*, **48**, 418 427.

van der Hoorn, A., Oldehinkel, A. J., Ormel, J., Bruggeman, R., Uiterwaal, C., & Burger, H. 2010. Non-right-handedness and mental health problems among adolescents from the general population: the Trails Study. *Laterality*, **15**, 304 316.

van Strien, J. W. 2003. The Dutch handedness questionnaire. Available from http://publishing.eur.nl/ir/repub/asset/956/PSY011.pdf.

Vayda, A. 1989. Explaining why Marings fought. Journal of Anthropological Research, 45, 159 177.

7

Handedness is associated with reproductive success in a nonindustrial society

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Abstract

The persistence of left-handedness in human populations has intrigued scientists for decades. Left-handedness is partially heritable and associated with health problems, which raises the question why natural selection has not driven it into extinction. Several authors suggest benefits for being left-handed. However, whether handedness really affects Darwinian fitness is unclear and not yet studied in a non-industrial society where selection pressures on health and handedness are likely to be similar to the situation in which handedness has evolved. We measured both hand preference and hand skill (speed and accuracy) of both hands, as they measure different aspects of handedness, and investigated their association with the number of offspring and self-reported illness in a non-industrial society in Papua, Indonesia. We found a significant positive association between strength of hand preference and number of children who died within the first two years of life. On the other hand men who showed strong lateralization in hand skill sired more children and showed a trend to have more children alive than weakly lateralized men. For women we found no such effects Our results indicate that strength of handedness, independent of direction, has fitness implications and that the persistence of the polymorphism in handedness may be ascribed to balancing selection, either as sexual antagonism, or as balancing selection of skill versus preference. No relationships between health and handedness were found, perhaps due to disease related selective disappearance of subjects with a specific handedness.

Introduction

Lateralization, the asymmetric distribution of function over the cerebral hemispheres, was long thought to be a human characteristic, but is now known to be a widely spread phenomenon throughout the animal kingdom. Population biases in behavioural lateralization have been found in many non-human species, but the distribution of the most apparent lateralized behaviour in humans, handedness, is exceptionally skewed, with only about 4-13% left-handers (Raymond *et al.* 1996; Perelle & Ehrman 1994). This skew has intrigued scientists for decades but a satisfactory explanation for this polymorphism found in every human population investigated thus far has not yet been provided.

Despite ample scope for environmental factors affecting the development of handedness (Schaafsma *et al.* 2009), heritability of handedness is substantial, varying between 0.23 and 0.66 (Denny & Sullivan 2007). This indicates that natural selection could act on handedness if handedness is not a neutral trait. Several studies have investigated the costs and benefits of left-, mixed- or right-handedness and results confirm that handedness is not a neutral trait. Non-right-handedness (in the literature sometimes classified as left- and/or mixed-handers) has been associated with possible fitness costs such as extremely low birth weights, delayed maturation (but see Eaton *et al.* 1996), and birth stress (for references see Searleman *et al.* 1989; Llaurens *et al.* 2009). The latter can lead to hypoxia and brain damage that potentially affects neurodevelopment (Bakan 1977; Ross *et al.* 1987; Coren & Halpern 1991; Miller *et al.* 2005). Non-right-handedness has also been associated with auto-immune diseases (Searleman & Fugagli 1987; Morfit & Weekes 2001).

Besides these disadvantages, some advantages for these groups have also been found. Non-right-handers are more prevalent among the top of interactive sport competitors suggesting that non-right-handers have increased chances of winning these interactions compared with right-handers and this may translate to winning aggressive interactions too (Raymond *et al.* 1996). Furthermore, they are reported to be more common among (instrumental) musicians (Byrne 1974; Peterson 1979; Götestam 1990; Hassler & Gupta 1993; Aggleton *et al.* 1994) and artists (Peterson 1979; Preti & Vellante 2007), and people of higher socio-economic status (higher income and position in companies; Faurie *et al.* 2008) possibly leading to benefits in sexual selection (Miller 2000; Nettle & Clegg 2006). In addition, handedness is often found to be associated with cognitive performance, although the results are ambiguous. Non-right-handedness has been found to be associated with lower cognition related skills by some (Miller 1971; Coren *et al.* 1981; Crow *et al.* 1998; Corballis *et al.* 2008; Siengthai *et al.* 2008; Rodriguez *et al.* 2010) but not by others (Hardyck *et al.* 1976; van der Elst *et al.* 2008). One study even found that healthy mixed- and left-handers are faster in a cognition related task compared to right-handers (Gunstad *et al.* 2007). Whether these associations translate into differential fitness in terms of reproductive success is unclear. McManus and Bryden (1992) reviewed the genetics of handedness and they found that parents of whom one was right-handed and one was left-handed reported to have fewer offspring than two right-handed parents and more than two left-handed parents (table 6 in McManus & Bryden 1992). Unfortunately, that observation could not be statistically tested. Faurie *et al.* (2006) investigated the association between handedness and reproductive success in French adults. They found an

interaction between direction of handedness and income, such that left-handed men with low income have the lowest number, and left-handed men with high income had the highest number of grandchildren relative to right-handed men.

Apart from the scarcity of data on reproductive success, three other aspects may contribute to the lack of understanding the persisting human polymorphism in handedness. First, many studies, like those of McManus and Bryden (1992) and Faurie et al (2006), have only investigated direction of lateralization. However, Nettle (2003) showed that the results of most studies finding right-handers to outperform left-handers in cognitive tasks are actually confounded by the fact that right-handers are generally more strongly lateralized than left-handers, and that it is strength of lateralization, independent of direction, that is associated with cognitive ability. This highlights that when investigating handedness attention should be paid to both direction and strength. Second, handedness can be measured in terms of preference (which hand is preferably used for a certain task) and in terms of performance or skill (is the task better performed with the right or the left hand) and both aspects are relatively independent as they do not show a strong correlation (Borod *et al*. 1984; Connolly & Bishop 1992; Steenhuis 1999; Doyen *et al*. 2008). Most studies investigating handedness in relation to health problems or number of offspring, have investigated hand preference, whereas it can as well be asymmetry of hand skill that is under natural selection pressures. Third, although both the costs and benefits of left-, mixed-, and right-handedness have received attention, all studies were performed in Western societies. These societies may no longer be under the selection pressures in which handedness has evolved.

In this paper we will investigate the association between both direction and strength of handedness and reproductive success, with handedness measured both in terms of preference and skill. Reproductive success of subjects was estimated based on the number of their children born, alive and deceased in the first two years of life. We also investigated whether the associations found are mediated through serious health problems. The study was carried out in a nonindustrial society in the highlands of Papua, Indonesia. We measured hand preference on ten ecologically relevant tasks and asymmetry of hand skill by means of a pegboard task, and accuracy in a ball throwing task.

Methods

Subjects

The Eipo people inhabit an area of about 150 km2 near the Eipomek river at approximately 4°25`-4°27` S, 140°00`-140°05` E. in the highlands of the Indonesian province of Papua, formerly known as Irian Jaya (New Guinea). The Eipo are horticulturists whose staple food consists of sweet potatoes and vegetables, complemented by the products of hunting, gathering and pig raising (Schiefenhövel 1976, 1991; personal observations, 2009). Because of the remoteness and inaccessibility of the area the Eipo valley is located in, it has until recently been isolated from the outside world. The first brief contact with Europeans was in 1959 during the expedition led by Gaisseau (Saulnier 1960), and more frequent contacts only began in 1974 when the interdisciplinary German Research Team began fieldwork there. Nowadays, the area is still accessible by foot or light aircraft only.

Western health care was absent until 2005 when a health centre was built which now offers basic health care.

The sample comprised 373 subjects (197 women and 176 men), and is a subsample of a larger dataset collected by the first two authors during a 3 months field survey executed in the three major villages of the valley in 2009-2010. Criteria for inclusion in this study was the minimum age, in this population, for women to give birth to their first born (18 years of age) and for men to sire their first born (20 years of age).

Our study was approved by the ethical committee of the Psychology Department of the University of Groningen. All subjects were recruited on a voluntary basis. As most subjects were illiterate they received verbal information about the survey in their local language (*Eipo yupe*) and those who asked for additional information before, during or after their participation were further informed. The subjects were informed about the possibility to abort their participation at any point in time. Those subjects that did not explicitly give their consent or did not want to participate were excluded from the study and not recorded, and those who did were recorded. This process was approved by the ethical committee.

Interviews: health status and reproduction

Each subject participated in a 30 minute interview lead by one of the two first authors who was assisted by one of three local assistants who translated between the Indonesian and the local language. After establishing a subject's name, we estimated the age of the subject as they do not record dates of births. Estimation of age was done by means of a series of major events in the community that the subject recollected, of which exact dates were known from documented records. Also the age of their first and last born was estimated and sometimes of other children too. When not all children's ages were estimated, the ages were estimated by interpolation based on the ages of their siblings.

Number of children born and number of children deceased were recorded, as was the age of the child when it deceased. As in multiple instances both parents volunteered and also information was obtained concerning and from siblings, the data obtained could be verified. The subjects were also questioned whether they themselves ever experienced severe (almost lethal) illness or injury. After the interview and the measurements of handedness (see below), the height of the subject was measured using a straight bough on which a scale was drawn, since height has been shown to correlate with reproductive success (e.g. Pawlowski *et al*. 2000).

Handedness preference measures

Hand preference was observed during an inventory consisting of 10 ecological relevant actions the subjects completed using the tools provided, and comprised both fine and gross motor skills. The tasks included: (1): one punch at a bag held up by an observer (mimicking giving someone a punch during a fight); (2): sharpening a wooden stick with a knife (hand used to handle knife was recorded); (3): hammering a wooden stick into the ground with a stone (hand used to handle stone was recorded); (4): machete use (imitating cutting vegetation); (5): throwing a small stone far away; (6): picking up a nut and putting it in the mouth; (7): picking up a small bead and handing it over to the observer; (8): drawing a circle on the ground with a wooden stick; (9): chasing away an imaginable

fly located on the subject's nose and (10): crushing a small stone with a big stone. The tasks comprised of both unimanual (nos. 1, 4, 5, 6, 7, 8, 9 and 10) and bimanual items (nos. 2 and 3). The tools were bimanually placed precisely in front of the subject in such a way that no cue for left- or right-hand use was given by a bias in the placement of tools. A score of -1 per task was given when performed with the left hand and +1 when performed with the right hand. Due to the very low sample size of individuals with a left hand preference (3 women and 7 men) direction of hand preference was omitted from the analyses (see below) and only strength of hand preference was investigated (see Schaafsma *et al.* 2011 for discussion about the low level of left-handedness in this population).

Asymmetry in hand skill measures

Pegboard task

To measure the speed of fine motor control of both hands a pegboard task was used. This task was based on the apparatus designed by Annett (p. 208 Annett 1985) and was constructed of hardwood (40 cm x 21 cm x 2.5 cm) with 2 parallel rows of 10 holes (rows 17 cm apart; holes spaced 3.75 cm apart each, Ø 0.5 cm, depth 1.2 cm). The pegboard was laid on a 50 x 40 cm plywood plate and placed on the lap of the subject who was sitting on a low flat rock. Ten steel pegs (Ø 0.45 cm, length 3 cm) were placed in the row of holes nearest to the subject. The subject fixated the apparatus in position with one hand, whereas the other performed the test (left and right hands in alternating order between the subjects). Subjects were instructed to move the pegs one by one to the equivalent hole at the other side of the pegboard, starting from the side of the hand used in the task. Each trial started with a practice trial moving 3 pegs forth and back. Next, the subject was instructed to move all pegs as fast as possible. The time to complete the task was recorded with a stopwatch. Subsequently the board was rotated and the procedure was repeated for the other hand. If a peg was dropped the trial was repeated for both hands.

Ball throwing task

To measure the accuracy of throwing of each hand, a ball throwing task was used. Subjects were standing 2.7 meters from a target (2 meters for a few elderly people lacking force) that was placed on eye height (approximately 1.5 meters). The target was a black cloth with bands printed on it. The white circle in the middle (Ø 7 cm) was surrounded by four circular bands (width 2.8 cm each and alternating black or white). The subjects were instructed to throw a tennis ball at the middle of the target and, if hit, rewarded with fifty points. Each adjacent circle was rewarded 10 points less compared to the band closer to the bull's eye. The zero band was imagined to be the adjoining band of 2.8 cm width of the outer printed band. If the ball landed outside the zero band the scores -10 to -50 were estimated by imagining the landing position onto the target area. In the rare event that the landing positions was further away than -50 (i.e. more than 34 cm from the zero band) -50 was recorded. Subjects performed 5 trials with each hand (start with left or right hand was alternated between individuals). The score of each throw was observed from a position just behind and slightly aside the participant and noted on a scale of -50 to +50.

Analyses

The dataset

The 197 women who participated in this study gave birth to 885 children of which 124 died (98 before their 2nd birthday) and 176 men sired 815 children of which 118 died (103 before their 2nd birthday) (Figure 1).

As was reported in earlier studies (Borod *et al.* 1984; Connolly & Bishop 1992; Steenhuis 1999; Doyen *et al.* 2008), the asymmetry of hand skill as measured with the pegboard task (L-R) and with the ball throwing task (R-L) correlated only weakly with hand preference (Spearman's r = 0.066 and r = 0.163, respectively). Out of 367 subjects 99 in the pegboard task and out of 369 subjects 153 in the ball throwing task showed better skill with the left hand than with the right hand.

Number of children born and alive

To model the relationship between the number of offspring born or surviving and handedness scores of the parents we corrected for parental age and age squared as the relationship between parental age and these two dependent variables was quadratic. We also included parent's height in the analyses as height is shown to be related to number of

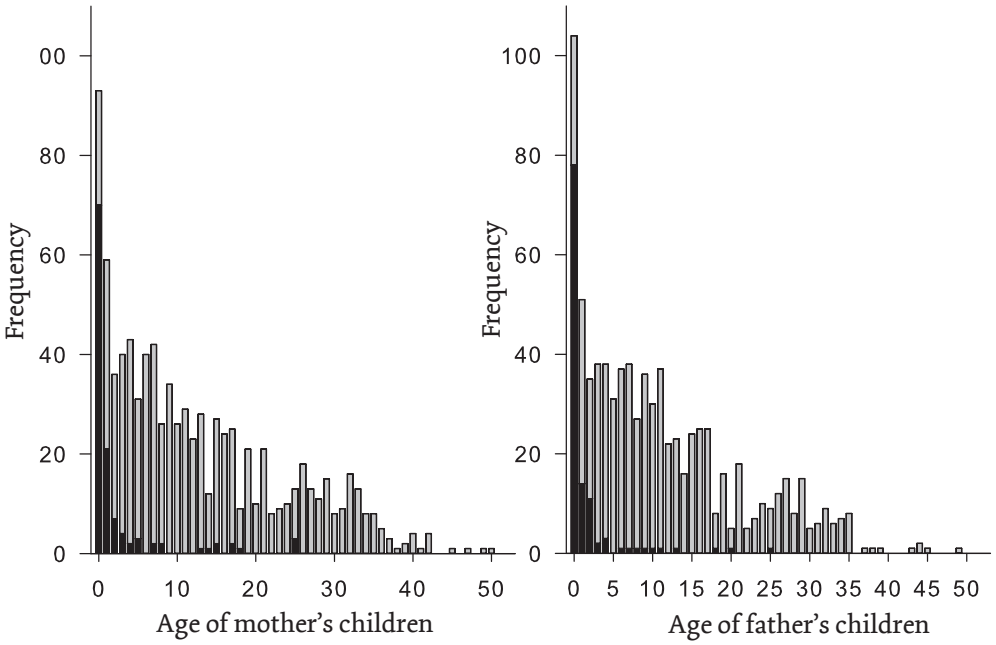

Figure 1 • Histograms of number of children per age group at the time of research (grey) and deceased within the first two years of life (black) of mothers (left panel) and fathers (right panel).

offspring (e.g. Pawlowski *et al.* 2000). The effect of handedness measures on the number of offspring, total born or still alive, was modelled with a negative binomial regression model with a logarithmic link (McCullagh & Nelder 1989) in the software program SPSS 16.0.

Due to the unexpected low sample size of individuals with a left hand preference (n=10) (see Schaafsma *et al.* 2011) we could not investigate the association between direction of hand preference and the number of offspring.

To analyze the predictive value of the strength of parental hand preference we used the following model: Number of offspring = age + age^2 + height + strength of hand preference. Strength of hand preference was defined as the absolute value of the total score on the handedness test battery for preference. The data concerning hand skill asymmetry (ball and pegboard tasks) were modelled: Number of offspring = age + age^2 + height + (score L+R) + direction of hand skill asymmetry (left or right) + strength of hand skill asymmetry + interaction between direction and strength of hand skill asymmetry. Following Nettle (2003) the left plus right hand score (L+R) score was incorporated to control for the overall performance in the task independent from the asymmetry. Direction of hand skill asymmetry was defined as 0 for individuals who were faster or more accurate with the left hand on the pegboard task or ball throwing task, respectively, and 1 for individuals who were faster or more accurate with the right hand. Strength of hand skill asymmetry was defined as the absolute value of the right hand score minus the left hand score (seconds in the pegboard and score of the ball throwing task). The interaction effect of strength times direction of handedness was included in the model to be able to differentiate between possible differential effects of strength of handedness between left- and right-handers on number of children. In order to interpret the main effects of direction and strength of handedness on the number of children the models were rerun without the interaction effect.

The analyses were performed for men and women separately because the response variables of men and women were not independent as in some cases both parents of the same children were included in this study, while the effects of handedness may be sex dependent. The fit of the models with the data was satisfactory, no overdispersion was present (scaled deviance/df <1.5 in all models).

Mortality of children in the first two years of life

In the first two years of life mortality is very high in this population (Figure 1). Therefore we investigated whether parental handedness influenced the survival chances in the first two years of life. In this analysis we had to consider the hierarchical structure of the data since the survival of children from the same parent may be not independent. We used two separate (fathers and mothers) two-level hierarchical logistic regression models (level 1 estimated variation in mortality at the child level, level 2 estimated variation at the parent level) using RIGLS (restricted iterative generalized least squares) estimation (MLWin 2.02, Rasbash *et al.* 2009) for binomial models as survival analysis. Again, the models were corrected for parental age and, when asymmetry of hand skill was addressed, total score on the tasks (L+R).

Self-reported illness

As we found a sex-specific effect of strength of handedness on reproductive success (see Table 1 and section Reproductive success) we investigated whether this effect could be mediated by individual's chance of ever having been exposed to severe illness. We used a logistic regression model for binary response variables (1: suffered from almost lethal illness; 0: did not suffer from almost lethal illness) in the software program SPSS 16.0. We included sex and controlled for the age of the subject, as older individuals would have had more time to have suffered from illnesses, and for total score on the tasks (L+R) when asymmetry of hand skill was addressed. The fit of the models with the data was satisfactory as no overdispersion was present (scaled deviance/df <1.5 in all models). These analyses were conducted on both sexes together. As no significant effect of sex was present, the models were not rerun per sex.

Results

Reproductive success

In females neither hand preference nor skill asymmetry had a statistically significant influence on any of our measures of reproductive success (Table 1). In contrast, in men several statistically significant associations between handedness and components of reproductive success were found (Table 1). A positive relationship was found between strength of hand skill as measured with the pegboard task and the number of children born (Figure 2). No relationship between child mortality during the first two years of life and this aspect of handedness was found in men. As to be expected on the basis of these results, strongly lateralized men, as measured with the pegboard task, showed a trend to have more children alive than weakly lateralized men, but this trend just did not reach statistical significance ($p = 0.066$, Table 1). Furthermore, men with a strong

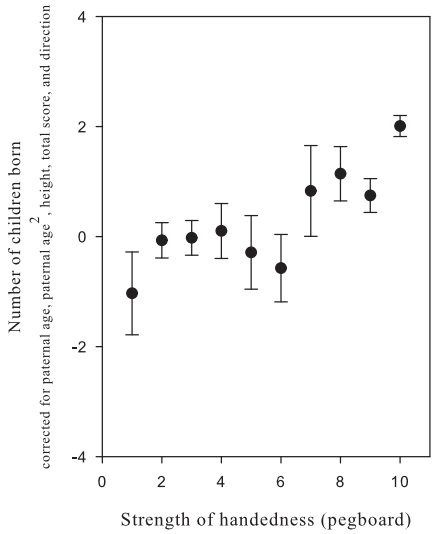

Figure 2 • The relationship in fathers between strength of hand skill, measured with the pegboard task and categorized in 10 groups of equal widths, and number of children born (mean and standard errors), corrected for paternal age, squared paternal age, height, total score on pegboard task (left hand + right hand) and direction of handedness as measured in the pegboard task.

Table 1 • Regression analyses of each association between different measures of handedness and components of reproductive success. Bs, standard errors, Wald statistics and p-values are presented. Results of the interaction effects are reported from the full models whereas those of the main effects are from models without the interaction effects.

		Hand preference					Pegboard task					Ball throwing task				
Variable		Predictor	B	SE	Wald	p	Predictor	B	SE	Wald	p	Predictor	B	SE	Wald	p
Number of children born																
	Men	Strength	0,144	0,392	0,136	0,713	Direction x Strength	-0,023	0,086	0,074	0,785	Direction x Strength	0,002	0,004	0,345	0,557
							Direction	-0,050	0,091	0,304	0,581	Direction	0,017	0,137	0,016	0,901
							Strength	0,074	0,032	5,260	0,022 *	Strength	-0,001	0,001	0,564	0,453
	Woman	Strength	-0,021	0,224	0,924	0,979	Direction x Strength	0,056	0,070	0,650	0,420	Direction x Strength	0,002	0,002	1,810	0,178
							Direction	0,010	0,081	0,015	0,902	Direction	-0,060	0,078	0,592	0,442
							Strength	-0,011	0,030	0,123	0,725	Strength	0,000	0,001	0,048	0,826
Number of children alive																
	Men	Strength	-0,232	0,366	0,402	0,526	Direction x Strength	-0,027	0,084	0,106	0,745	Direction x Strength	0,002	0,004	0,409	0,522
							Direction	-0,072	0,090	0,639	0,424	Direction	0,028	0,135	0,042	0,838
							Strength	0,060	0,032	3,378	0,066 (*)	Strength	-0,001	0,001	0,553	0,457
	Woman	Strength	-0,006	0,243	0,001	0,981	Direction x Strength	0,057	0,075	0,581	0,446	Direction x Strength	0,129	0,569	0,051	0,821
							Direction	-0,004	0,087	0,002	0,963	Direction	-0,062	0,244	0,064	0,801
							Strength	-0,004	0,032	0,014	0,906	Strength	0,013	0,253	0,002	0,960
Number of children deceased in the first two years of life																
	Men	Strength	6,451	2,533	6,506	0,011 *	Direction	-0,025	0,291	0,007	0,933	Direction	-0,259	0,425	0,373	0,541
							Strength	0,094	0,090	1,088	0,297	Strength	0,000	0,002	0,052	0,820
							Direction x Strength	-0,291	0,298	2,232	0,526	Direction x Strength	0,004	0,012	0,597	0,897
	Women	Strength	-0,746	0,635	1,380	0,240	Direction	-0,246	0,208	1,396	0,237	Direction	-0,077	0,197	0,152	0,697
							Strength	-0,111	0,077	2,100	0,147	Strength	-0,001	0,002	0,421	0,516
							Direction x Strength	-0,195	0,264	3,227	0,358	Direction x Strength	-0,001	0,005	0,503	0,918

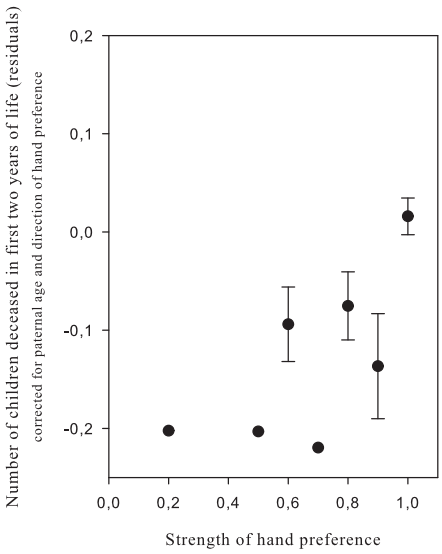

Figure 3 • The relationship in fathers between strength of hand preference, and number of children deceased in the first two years of life (mean and standard errors), corrected for paternal age and direction of hand preference

Table 2. Regression analyses of the associations between different measures of handedness and self-reported illness Bs, standard errors, Wald statistics and p-values are presented. For further details see table 1

Hand preference					**Pegboard task**					**Ball throwing task**				
Predictor	B	SE	Wald	p	Predictor	B	SE	Wald	p	Predictor	B	SE	Wald	p
Strength x Sex	-1,603	2,070	0,600	0,439	Strength x sex	-0,291	0,202	2,082	0,149	Strength x Sex	0,002	0,004	0,160	0,689
Strength	0,101	1,015	0,010	0,921	Strength	-0,084	0,105	0,639	0,424	Strength	-0,004	0,002	3,245	0,072 (*)
Sex	0,409	0,251	2,663	0,103	Sex	0,331	0,253	1,714	0,190	Sex	0,388	0,257	2,269	0,132

hand preference had significantly more children that died before the age of 2 (Figure 3). However, this did not significantly affect the number of children alive. No relationships between handedness as measured with the ball throwing task and components of reproductive success were found (Table 1).

Remarkably, eight out of the nine associations between the strength of handedness and proxies for reproductive success for men and women were opposite in direction (binomial test: p = 0.030).

Self-reported illness

26.3% of the subjects reported to have suffered at least once from a severe illness during their lifetime. Weakly lateralized subjects, as measured with the ball throwing task, showed a non-significant trend (p = 0.072) to have suffered less from a severe illness (Table 2). Other associations were far from statistically significant.

Discussion

This study presents data on the association between direction and strength of handedness and reproductive success in a nonindustrial society. We studied both asymmetry of skill of the hands and hand preference and as these two only weakly correlated (Borod *et al.* 1984; Connolly & Bishop 1992; Steenhuis 1999; Doyen *et al.* 2008; this study), these two measures probably reflect two different facets of lateralization and will therefore be discussed separately.

Hand preference

We found in men that strength of hand preference was significantly and positively associated with the number of children who died within the first two years of life. However, this did not result in a negative relationship between strength of hand preference and number of offspring alive. Neither was there a positive relationship between strength of hand preference and number of children sired. The discrepancy can be caused by a reduction of statistical power in the latter two analyses. Since the majority of children survived, the number of deceased children does only marginally affect the number of surviving children. In addition, although the B-value for the number of children sired is positive, it is considerably smaller than the negative B-value for the number of children survived (Table 1). Although the latter is not significant, the effect size might not be irrelevant for natural selection over many generations. We cautiously conclude therefore that men with a relatively strong hand preference may have a fitness disadvantage due to a higher number of children that died in the first two years of life.

Our sample consisted of a surprisingly low number of individuals with a left hand preference (3 women and 7 men, for a discussion of this finding see chapter 6). Therefore, an effect of direction of preference on number of children could not be investigated. Thus, we can not support or oppose the observation of McManus and Bryden (1992) and Faurie *et al.* (2006) concerning direction of handedness and reproductive success. However, as in general left-handers are less strongly lateralized than right-handers (e.g. Nettle 2003), it may well be possible that in the studies of Faurie et al (2006) and McManus and Bryden (1992), who only had information on direction and not on strength of handedness, it was actually strength, like in our study, and not direction of handedness that was underlying their observed association between handedness and reproductive success.

Asymmetry of hand skill

In contrast to associations concerning the direction of hand preference, associations concerning the direction of hand skill could be reliably investigated as sample sizes of both groups (performing better with left hand or performing better with right hand) were substantial. We found no associations between direction of lateralization and reproductive success, suggesting that direction of lateralization does not affect Darwinian fitness. Like was the case for hand preference, we did find significant associations concerning the strength of asymmetry of hand skill. Strongly lateralized men, as measured in the pegboard task, sired more children and showed a trend to have more living children than weakly lateralized men. Therefore, we conclude that strength of lateralization in skill, independent of direction, is positively associated with reproductive success. Since this

was found for the pegboard and not for the ball throwing task the results suggests that lateralization of fine motor skills is most strongly under natural selection.

Persistence of polymorphism in handedness

We found that strong lateralization in hand skill is an advantageous trait in men. Apparently natural selection has however not rendered weak lateralization to go extinct as we still find weakly lateralized individuals in this nonindustrial society. We did not have the opportunity to investigate subsequent generations and therefore we do not have the full picture concerning the fitness of our subjects. Hence, we can not be certain that eventually both strongly and weakly lateralized individuals yield the same Darwinian fitness revenues. However, the differential reproductive success found in this study between strongly and weakly lateralized individuals reported in our study may be accurate and can be maintained due to sexual antagonism. When a trait has negative effects in one sex it can persist in a population when it is advantageous in the other sex. Although we found no significant associations between strength of lateralization and reproductive success in women, we did find that eight out of nine associations between strength of handedness and reproductive success were opposite in direction for men and women, suggesting sexual antagonism. Natural selection favouring strong lateralization in hand skill in men may thus be confined due to possible detrimental effects on reproductive success of women. As men show more variation in reproductive success than women, larger sample sizes are needed to find associations between traits and reproductive success in women than in men. Such studies may shed light on the possibility of sexual antagonism driving the persistence of different phenotypes of left-handedness.

Our results indicate that for men it is advantageous to be strongly lateralized on skill, but detrimental to have a strong hand preference. This seemingly contrasting result could also play a role in the persistence of different phenotype of handedness. Although the correlation between hand preference and asymmetry in hand skill is low, it is significant and asymmetry of skill is thus to a small degree positively related to the preference to use the hand with the better performance. As strong hand preference may have detrimental effects this could result in a form of balancing selection possibly leading to the persistence of the different phenotypes of handedness. Furthermore, the differential selection pressures on asymmetry of hand skill and hand preference may be the underlying reason of the weak correlation between the two facets of handedness.

In order to examine whether the associations we found between strength of lateralization and reproductive success is mediated by the health of individuals we also investigated whether lateralization affected the chances of individuals to ever having experienced a severe illness. We included sex as the effects of lateralization on fitness were different for men and woman. Lateralization did not in any way affect risk of severe illness, although in one case (lateralization of ball throwing) the p-value was lower than 0.1. However, lateralization of ball throwing did not display any relationship with fitness aspects. This suggests that the associations we found between strength of lateralization and reproductive success are not mediated by ever having experienced a severe illness.

Many studies have found a relationship between handedness and illnesses (see introduction) and the reason we did not find such an association may be explained in two ways. Individuals suffering from severe diseases may have died from these diseases,

resulting in selective disappearance if handedness and health are associated (Chapter 8, this thesis). Additionally, as no written records of health history were available and we had to rely on self-reported data, we could not differentiate between different kinds of illnesses, nor evaluate their severeness.

Our study shows that handedness should not be investigated solely in terms of direction, but also in terms of strength. Although this study lacks power concerning the effect of direction of hand preference due to low numbers of left-handed individuals, we did have a large data set for analysing the effect of direction of hand skill on fitness. However, even in this case predictive factors on fitness proxies did only entail strength and not direction of handedness. Our results open new research avenues for the study of the persistence of variation of handedness. Studies focussing on the differential fitness revenues of handedness between the sexes, and between hand preference and skill will shed more light on the possibility of these specific forms of balancing selection being the mechanism underlying the different phenotypes of handedness.

References

Aggleton, J.P., Kentridge, R.W. & Good, J.M.M. 1994. Handedness and Musical Ability: A Study of Professional Orchestral Players, Composers, and Choir Members. *Psychology of Music*, **22(2)**, 148-156.

Annett, M. 1985. Left, Right, Hand and Brain: The Right Shift Theory. Erlbaum., Hillsdale, NJ.

Bakan, P. 1977. Left handedness and birth order revisited. *Neuropsychologia*, **15(6)**, 837–839.

Borod, J., Caron, H. & Koff, E. 1984. Left-handers and right-handers compared on performance and preference measures of lateral dominance. *British journal of Psychology*, **75**, 177-186.

Byrne, B. 1974. Handedness and musical ability. *British Journal of Psychology*, **2**, 279-281.

Connolly, K.J. & Bishop, D.V. 1992. The measurement of handedness: a cross-cultural comparison of samples from England and Papua New Guinea. *Neuropsychologia*, **30(1)**, 13-26.

Corballis, M.C., Hattie, J. & Fletcher, R. 2008. Handedness and intellectual achievement: an even-handed look. *Neuropsychologia*, **46(1)**, 374-378.

Coren, S. & Halpern, D.F. 1991. Left-handedness: A marker for decreased survival fitness. *Psychological Bulletin*, **109(1)**, 90–106.

Coren, S., Porac, C. & Duncan, P. 1981. Lateral preference behaviors in preschool children and young adults. *Child Development*, **52(2)**, 443–450.

Crow, T., Crow, L., Done, D. & Leask, S. 1998. Relative hand skill predicts academic ability: global deficits at the point of hemispheric indecision. *Neuropsychologia*, **36(12)**, 1275-1282.

Denny, K. & Sullivan, V.O. 2007. The economic consequences of being left-handed: Some sinister results. *Journal of Human Resources*, **42(2)**, 353.

Doyen, A.-L., Dufour, T., Caroff, X., Cherfouh, A. & Carlier, M. 2008. Hand preference and hand performance: cross-sectional developmental trends and family resemblance in degree of laterality. *Laterality*, **13(2)**, 179-197.

Eaton, W., Chipperfield, J. & Ritchot, K. 1996. Is a maturational lag associated with left-handedness? A research note. *Journal of Child Psychology and Psychiatry*, **37(5)**, 613-617.

Van der Elst, W., Van Boxtel, M.P.J., Van Breukelen, G.J.P. & Jolles, J. 2008. Is left-handedness associated with a more pronounced age-related cognitive decline? *Laterality*, **13(3)**, 234-254.

Faurie, C., Alvergne, A., Bonenfant, S., Goldberg, M., Hercberg, S., Zins, M. & Raymond, M. 2006. Handedness and reproductive success in two large cohorts of French adults. *Evolution and Human Behavior*, **27(6)**, 457–472.

Faurie, C., Bonenfant, S., Goldberg, Marcel, Hercberg, Serge, Zins, Marie & Raymond, M. 2008. Socio-economic status and handedness in two large cohorts of French adults. *British Journal of Psychology*, **99(4)**, 533–554.

Gunstad, J., Spitznagel, M.B., Luyster, F., Cohen, R. a & Paul, R.H. 2007. Handedness and cognition across the healthy lifespan. *The International Journal of Neuroscience*, **117(4)**, 477-485.

Götestam, K. 1990. Lefthandedness among students of architecture and music. *Perceptual and Motor Skills*, **70(3)**, 1323-1327.

Hardyck, C., Petrinovich, L. & Goldman, R. 1976. Left-handedness and cognitive deficit. *Cortex*, **12(3)**, 266-279.

Hassler, M. & Gupta, D. 1993. Functional brain organization, handedness, and immune vulnerability in musicians and non-musicians. *Neuropsychologia*, **31(7)**, 655–660.

Llaurens, V., Raymond, M. & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 881-894.

McCullagh, P. & Nelder, J. 1989. Generalized linear models. Monographs on statistics and applied probability. 2nd ed., London: Chapman & Hall.

McManus, I. & Bryden, M. 1992. The genetics of handedness, cerebral dominance, and lateralization. In I. Rapin & S. Segalowitz, eds. *Handbook of Neuropsychology*. Amsterdam: Elsevier Science, pp. 115-144.

Miller, E. 1971. Handedness and the pattern of human ability. *British Journal of Psychology*, **62(1)**, 111-112.

Miller, G. 2000. The mating mind: how mate choice shaped the evolution of human nature, New York: Doubleday.

Miller S.P., Ferriero D.M., Leonard C., Piecuch R., Glidden D.V., Partridge J.C., Perez M., Mukherjee P., Vigneron D.B., Barkovich A.J. 2005. Early brain injury in premature

newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. *The Journal of Pediatrics*, **147(5)**, 609-616.

Morfit, N. & Weekes, N. 2001. Handedness and immune function. *Brain and Cognition*, **46(1-2)**, 209–213.

Nettle, D. 2003. Hand laterality and cognitive ability: A multiple regression approach. *Brain and Cognition*, **52(3)**, 390-398.

Nettle, D. & Clegg, H. 2006. Schizotypy, creativity and mating success in humans. *Proceedings of the Royal Society B: Biological Sciences*, **273(1586)**, 611-615.

Pawlowski, B., Dunbar, R. & Lipowicz, A. 2000. Tall men have more reproductive success. *Nature*, **403(6766)**, 156-156.

Perelle, I.B. & Ehrman, L. 1994. An international study of human handedness: the data. Behavior genetics, 24(3), 217-227.

Peterson, J. 1979. Left-handedness: differences between student artists and scientists. *Perceptual and Motor Skills*, **48(3 Pt 1)**, 961-962.

Preti, A. & Vellante, M. 2007. Creativity and psychopathology: higher rates of psychosis proneness and nonright-handedness among creative artists compared to same age and gender peers. *The Journal of Nervous and Mental Disease*, **195(10)**, 837-845.

Rasbash, J., Charlton, C., Browne, W.J., Healy, M. & Cameron, B. 2009. MLwiN Version 2.21.

Raymond, M., Pontier, D., Dufour, A.B. & Møller, A.P. 1996. Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society: Biological Sciences*, **263(1377)**, 1627–1633.

Rodriguez, A., Kaakinen, M., Moilanen, I., Taanila, A., McGough, J.J., Loo, S. & Järvelin, M.-R. 2010. Mixed-handedness is linked to mental health problems in children and adolescents. *Pediatrics*, **125(2)**, 340-348.

Ross, G., Lipper, E.G. & Auld, P.A. 1987. Hand preference of four-year-old children: its relationship to premature birth and neurodevelopmental outcome. *Developmental medicine and child neurology*, **29(5)**, 615-622.

Saulnier, T. 1960. Les Papous coupeurs de têtes: 167 jours dans la préhistoire. Editions du Pont Royal: Paris.

Schaafsma, S., Geuze, R.H., Riedstra, B., Schiefenhövel, W., Bouma, A. & Groothuis, T. 2011. Handedness in a non-industrial society challenges the fighting hypothesis as an evolutionary explanation of left-handedness. *Evolution & Human Behavior*, In press.

Schaafsma, S., Riedstra, B.J., Pfannkuche, K.A., Bouma, Anke & Groothuis, T. 2009. Epigenesis of behavioural lateralization in humans and other animals. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 915-927.

Schiefenhövel, W. 1976. Die Eipo-Leute des Berglands von Indonesisch-Neuguinea: Kurzer Überblick über den Lebensraum und seine Menschen. Einführung zu den Eipo-Filmen des Humanethologischen Filmarchivs der Max-Planck-Gesellschaft. *Homo*, **26(4)**, 263-275.

Schiefenhövel, W. 1991. Eipo. In T. Hays, ed. *Encyclopedia of World Cultures, Volume II*. Boston: G.K.Hall & Co, pp. 55-59.

Searleman, A. & Fugagli, A. 1987. Suspected autoimmune disorders and left-handedness: evidence from individuals with diabetes. Crohn's disease and ulcerative colitis. *Neuropsychologia*, **25(2)**, 367-374.

Searleman, A., Porac, C. & Coren, S. 1989. Relationship between birth order, birth stress, and lateral preferences: a critical review. *Psychological Bulletin*, **105(3)**, 397-408.

Siengthai, B., Kritz-Silverstein, D. & Barrett-Connor, E. 2008. Handedness and cognitive function in older men and women: a comparison of methods. *The Journal of Nutrition, Health & Aging*, **12(9)**, 641-647.

Steenhuis, R.E. 1999. The relation between hand preference and hand performance: what you get depends on what you measure. *Laterality*, **4(1)**, 3-26.


ACETAMOL
500 mg
Ántalgin
500 mg
1000 kapsul
TETRACYCLINE
250 mg
OXYTETRACYCLINE

8

Health care explains variation in left-handedness

Sara M Schaafsma & Ton G G Groothuis • Manuscript

Abstract

A minority of left-handers is present in all societies investigated. Left-handedness is a heritable trait and associated with Darwinian fitness cost. An evolutionary hypothesis explaining the persistence of left-handedness is the fighting hypothesis, which postulates that left-handers have a frequency-dependent benefit in fights. This was supported by the finding of a positive correlation between the proportion of left-handedness and homicide in 12 nonindustrial human societies. However, our recent study challenged this idea and since left-handedness is associated with health problems we investigate whether public expenditure on health care is a better explanatory factor for the variation among societies in proportion of left-handedness. Public expenditure on health care was a significant explanatory factor and explained variation in proportions of left-handedness significantly better than the number of homicides. This suggests that the persistence of left-handedness is at least partly the by-product of pathologies on which selection pressures can not act upon. Our study provides a new framework for explaining the development, function and evolution of human left-handedness, having also consequences for the study of behavioural lateralization in other animal species

Introduction

Left-handedness is present in all societies investigated with low relative frequencies of around 10 % (Cashmore *et al.* 2008). Left-handedness is a heritable trait and associated with fitness cost (see references in Llaurens *et al.* 2009). This led behavioural ecologists and psychologists to speculate on the intriguing question of why left-handedness has not yet gone extinct. One explanation is provided by the fighting hypothesis (Raymond *et al.* 1996), proposing that left-handers have a frequency-dependent advantage in fights, since being the rarer phenotype may increase winning chances as almost all people have predominantly experience with right-handers. This can results in higher chances of left-handers to survive a fight, and also increase their fitness indirectly by an increase in dominance and social rank which can in turn positively affect female mate choice.

Support for this theory was twofold. First, Raymond and colleagues (Raymond *et al.* 1996) found that in physical interactive sports (e.g. fencing, tennis) the percentage of left-handers is substantially increased compared to non-physical interactive sports (e.g. gymnastics). Second, in a cross-cultural study among non-industrial societies, Faurie and Raymond found that when homicide levels are elevated, making winning fights more important, the percentage of left-handedness is increased concurrently (Faurie & Raymond 2005). However, most of the handedness data used in this studies were either based on film material not intended for laterality research or on indirect measures only and recently it was found that the Eipo, one of the societies showing very high levels of left-handedness and homicide in the study by Faurie and Raymond (Faurie & Raymond 2005), did actually show low levels of left-handedness when they were tested for handedness directly and in more detail (Schaafsma *et al.* 2011). When the data of the cross-cultural study by Faurie and Raymond (Faurie & Raymond 2005) were corrected for our estimation of left-handedness in this particular population (Schaafsma *et al.* 2011), the original correlation between homicide and left-handedness was no longer significant (Spearman's correlation coefficient r_s= 0.83, p = 0.01 to r_s = 0.643, p = 0.09) undermining the fighting hypothesis.

Left-handedness is at least to a certain extent associated to possible Darwinian fitness costs (e.g. low birth weight: Powls et al 1996, Elia et al 2007; intellectual disabilities: Grouios *et al.* 1999; developmental disorders: Geschwind and Galaburda 1985, Gangestad and Yeo 1997; and higher accident proneness: Daniel & Yeo 1994), and can provide an alternative explanation for the persistence of left-handedness. Increased selection against pathologies in these non-industrial societies lacking elaborate health care, and therefore indirectly also against left-handedness, could explain the lower levels of left-handedness found in the Eipo society (Schaafsma *et al.* 2011). Furthermore, McManus and colleagues (2010) showed that left-handedness was about 3% between 1880 and 1900. The rise of percentages of left-handers in Western societies following this period may have been a result of the professionalization of public health that started in Britain around the latter half of the 19th century (Starr 2009).

To further investigate whether the fighting or health care hypothesis provide the best explanation for variation in levels of left-handedness we examined the relationship

of homicide rate and health care with percentages of left-handedness among Western societies. Percentages of left-handers vary substantially among Western populations (Cashmore et al 2008; McManus et al 2010). This variation, together with variation in homicide and health care intensity provides opportunity to investigate whether the fighting or health care hypothesis can best explain the levels of left-handedness in Western populations.

Methods

Handedness measures

Data on handedness was obtained from the 1994 study of Perelle and Ehrman (Perelle & Ehrman 1994) who reported handedness scores, obtained from the same handedness questionnaires, from 12 Western countries (Australia, Belgium, Canada, UK, France, Italy, Mexico, The Netherlands, New Zealand, Spain, Turkey, USA). Respondents categorized their handedness based on self perception (strongly left-handed, moderately left-handed, ambidextrous, moderately right-handed and strongly right-handed) and also indicated their writing hand. Our analyses were performed on both self-perceived left-handedness and left hand writing.

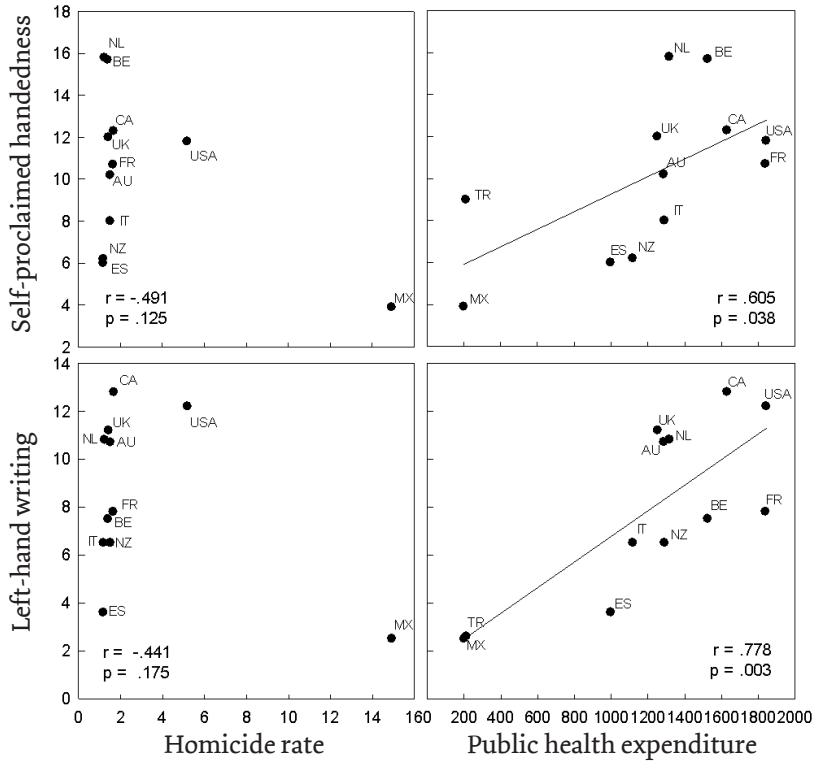

Figure 1. Percentages of left-handedness (self-proclaimed handedness (upper panels), left-hand writing (lower panels)) as a function of homicide rate (left) and public health (right) in Western societies. Homicide rate and public health are from the year 1998.

Homicide measure

Numbers on total recorded intentional successful homicides (and its percentage committed with a firearm) were taken from the United Nations Survey of Crime Trends and Operations of Criminal Justice Systems. We used data of the year 1998 as this is the year in which data were available of all countries reported in the Perelle and Ehrman study, except for Belgium, and closest to the year handedness measurements were conducted in. Data concerning Belgium was taken of 1995 (a year that data of 3 countries reported in the Perelle and Ehrman study were missing and was therefore not used as the main resource of data concerning homicide). Turkey was not included in the models as no data were available for both years. This yielded a sample size of 11. Correlations of the variable over time was very high (homicide rate in 1998 - latest available year 2006 (Italy, Mexico and The Netherlands) or 2008 (all other countries): $r = 0.989$, $n = 11$, $p<0.001$). Furthermore, the analyses were repeated omitting the United States of America and Mexico as homicides committed with a firearm were extreme outliers (1998: 3.37 (USA) and 3.45 (Mexico) compared to mean = 0.24, stand error = 0.07 stand dev = 0.165 for all other countries known (Spain, UK, New Zealand, Australia, Canada)) and the use of fire arms are likely to be irrelevant to test the fighting hypothesis.

Health measures

Data concerning public expenditure on health care (US$ PPP) in 1998 (except for Belgium, data was not available for that year, and data of 1996 was used) were taken from the Organisation for Economic Co-operation and Development (http://stats.oecd.org). This yielded a sample size of 12. Correlations of the variable over time was very high (health care 1995-2007 (excluding NL as no data was available): $r = 0.748$, $n = 11$, $p = 0.008$).

Statistics

Using SPSS 16, we applied linear regression models to investigate the explanatory power of the different variables. As no differences in the number of variables were used we could simply compare the R^2 among the different models. Using Microsoft Office Excel 2003, a Steiger's Z-test for correlated correlations (Steiger 1980) was executed to test whether the fighting and health care hypotheses significantly differed from each other. This test requires the same sample size on both variables, so data of Turkey was omitted resulting in a sample size of 11.

Results

A significant positive correlation was found between public expenditure on health care (US$ PPP) and level of handedness (both self-proclaimed left-handedness and left-hand writing) whereas a no correlation was found between total recorded intentional successful homicides and handedness measures (figure 1). The R^2 in the model with homicide as predictor decreased for self-proclaimed handedness and increased for left-hand writing (and the r-values of both models changed from negative to positive) when USA and Mexico were omitted due to their extreme incidence of fire arm induced homicide ($R^2 = 0.073$, $p = 0.482$ $R^2 = 0.256$, $p = 0.165$ respectively) and remained far from significant. The

correlation between handedness and health care significantly differed from the correlation between handedness and homicide rate (self-proclaimed handedness $Z = -2.19$, $n = 11$, $p = 0.028$; left-hand writing $Z = -2.18$, $n = 11$, $p = 0.029$). This difference disappeared when the USA and Mexico were taken out of the model ($Z = -0.92$, $n = 9$, $p = 0.358$ and $Z = 0.428$, $n = 9$ $p = 0.668$ respectively) possibly due to the decreased sample-size.

Discussion

The persistent low frequency of left-handedness in all human populations has intrigued scientists for decades. Since recent data from a non-industrial society undermine the importance of the fighting hypothesis (Schaafsma *et al.* 2011/ chapter 6, this thesis) we proposed, as an alternative, the health care hypothesis, explaining variation in left-handedness by variation in health care, as left-handedness may at least partially be a by-product of health problems (see introduction). When selection pressures against pathologies relax due to expanding health care in Western societies it can be expected that the percentage of left-handedness will increase concurrently. Indeed we found a positive correlation between percentage of left-handedness and public expenditure on health care. Moreover, public expenditure on health care was a significantly better explanatory factor for variation in left-handedness than the number of homicides in the society (in which the r-values of these models were even negative) therefore favouring the health care hypothesis over the fighting hypothesis. The correlation of public expenditure on health care between years was very high indicating that these findings are robust.

The finding that left-handers are overrepresented in interactive sports (Raymond *et al.* 1996) has been used to support the fighting hypothesis. However, this finding can be caused by the possibility that left-handers are more aggressive, as has been demonstrated for soccer players (Dane & Sekertekin 2005), or have better motor skills (reviewed in Harris 2010). For these reasons left-handers may be more likely to perform interactive sports and can therefore increase in frequency even without a frequency-dependent benefit. However, even when a frequency-dependent advantage for left-handers in fights does exist, this seems to be unlikely to be the evolutionary force explaining current percentages of left-handedness in Western societies as McManus and colleagues showed that percentages of left-handedness rose since 1900 (McManus *et al.* 2010). Faurie and Raymond stated that in Western societies "the level of violence has dramatically changed, as well as the type of violence (predominant use of long-range and powerful weapons, which probably do not offer a particular advantage to left-handers)" (Faurie & Raymond 2005). Based on this assumption it would be expected that, following the decrease of the advantage for left-handers in fights, the percentage of left-handers would decrease over time. This is in contrast to the increase of percentages of left-handedness over time since 1900 (McManus *et al.* 2010), undermining the fighting hypothesis. This rise could however be explained by the professionalization of public health that started in Britain around the latter half of the 19th century (Starr 2009).

The higher levels of left-handedness before 1880 in Western societies as reported by McManus and colleagues (2010) could theoretically be explained by the fighting

hypothesis. Some support for this theory could be found in the fact that when we analysed our data set without two countries with high rates of homicide conducted with a firearm the R^2 of the model explaining left hand writing increased. However, it did never equal the R^2 values of the models with health care as an explanatory variable. Furthermore, the R^2 of the model explaining self-proclaimed handedness decreased when we analysed the data set without the two countries with high rates of homicide conducted with a firearm.

In conclusion, although the frequency-dependent benefit may perhaps explain the overrepresentation of left-handers in interactive sports, we believe it is the association between left-handedness and health problems that evolution could not completely select against that is the driving force underlying the persistence of left-handedness in human populations.

References

Cashmore, L., Uomini, N. & Chapelain, A.S. 2008. The evolution of handedness in humans and great apes: a review and current issues. *Journal of Anthropological Sciences*, **86**, 7–35.

Dane, S. & Sekertekin, M.A.L.I. 2005. Differences in handedness and scores of aggressiveness and interpersonal relations of soccer players. *Perceptual and Motor Skills*, **100**, 743-746.

Daniel, W.F. & Yeo, R. a, 1994. Accident proneness and handedness. *Biological Psychiatry*, **35(7)**, 499.

Elia, M., Mulligan, J. & Betts, P. 2007. Relationship between birth weight and handedness. *Proceedings of the Nutrition Society*, **66**(Special issue), 3a.

Faurie, C. & Raymond, M. 2005. Handedness, homicide and negative frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, **272(1558)**, 25-28.

Gangestad, S. & Yeo, R. 1997. Behavioral genetic variation, adaptation and maladaptation: an evolutionary perspective. *Trends in Cognitive Sciences*, **1(3)**, 103-108.

Geschwind, N. & Galaburda, A.M. 1985. Cerebral lateralization - Biological mechanisms, associations, and pathology: I-III A hypothesis and a program for research. *Archives of Neurology*, **42**, 428–459; 521–552; 634–654.

Grouios, G., Sakadami, N., Poderi, a & Alevriadou, a, 1999. Excess of non-right handedness among individuals with intellectual disability: experimental evidence and possible explanations. *Journal of Intellectual Disability Research*, **43(4)**, 306-13.

Hardyck, C. & Petrinovich, L.F. 1977. Left-handedness. *Psychological Bulletin*, **84(3)**, 385-404.

Harris, L.J. 2010. In fencing, what gives left-handers the edge? Views from the present and the distant past. *Laterality*, **15(1)**, 15-55.

Llaurens, V., Raymond, M. & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 881-894.

McManus, I., Moore, J., Freegard, M. & Rawles, R. 2010. Science in the Making: Right Hand, Left Hand. III: Estimating historical rates of left-handedness. *Laterality*, **15(1/2)**, 186-208.

Perelle, I.B. & Ehrman, L. 1994. An international study of human handedness: the data. *Behavior Genetics*, **24(3)**, 217-227.

Powls, A., Botting, N., Cooke, R. & N, 1996. Handedness in very-low-birth weight (VLBW) children at 12 years of age: relation to perinatal and outcome variables. *Developmental Medicine and Child Neurology*, **38(7)**: 594-602

Raymond, M., Pontier, D., Dufour, A.B. & Møller, A.P. 1996. Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society - Biological Sciences*, **263(1377)**, 1627–1633.

Schaafsma, S., Geuze, R.H., Riedstra, B., Schiefenhövel, W., Bouma, A. & Groothuis, T. Handedness in a non-industrial society challenges the fighting hypothesis as an evolutionary explanation of left-handedness. *Evolution & Human Behavior*, In press.

Starr, P. 2009. Professionalization and public health: historical legacies, continuing dilemmas. *Journal of Public Health Management and Practice*, **15(6)**, S26-30.

Steiger, J.H. 1980. Tests for comparing elements of a correlation matrix. *Psychological Bulletin*, **87(2)**, 245-251.

9

General discussion

Sara M Schaafsma

Abstract

In this thesis I aimed to contribute to the understanding of lateralization by investigating both the proximate and the ultimate mechanisms underlying this phenomenon. Lateralization is a fundamental aspect in the organization of brain and behaviour, but there is ample variability in both direction (right versus left) and strength (independent of direction) of lateralization between individuals and species. In order to fully understand the existence of a trait such as lateralization one has to answer all of Tinbergen's "four questions" concerning function, evolution, causation and development. The first two address ultimate explanations at the species level; the latter two address the proximate explanations at the individual level. Although this thesis did not answer all four questions to the full extent it contributed to answers to all of them.

Proximate factors underlying lateralization

The first part of this thesis addressed the proximate questions concerning lateralization. In chapter 2 the literature on the ontogeny of lateralization was reviewed. Insight into the ontogeny of lateralization is highly relevant to the understanding of both its evolution and the possible constraints it may have on plasticity as well as its adaptive flexibility. Lateralization is a heritable trait and scientists have modelled this heritability and presented various genetic models. However, the extent of the genomic component in the inheritance pattern is still unclear. Although, for example, cross-fostering studies suggest that handedness is a heritable trait, this trait is, at least in non-human primates, transferred by non-genomic heritability. After reviewing the predominant genetic models concerning lateralization, and some of the challenges to them, such as the existence of monozygotic twins that are discordant for handedness and sex differences in frequency of left-handedness in humans, and after discussing the experimental work performed in non-human animals, chapter two concluded that there are ample indications that environmental factors influence the individual development of lateralization. Although in humans predispositions for handedness are already present in the womb, handedness is still open to environmental influences later in life, and in chapter two these environmental effects were subsequently reviewed. Head position in human babies seems to be causative to handedness. In addition, head position in the egg in birds may also be causative to lateralization, but this effect is not yet disentangled from effects of asymmetric light input in the eyes due to the fact that one eye faces the translucent egg shell, whereas the other faces the bird's body. Although it is known that this asymmetric light input increases lateralization in birds (Rogers 1996), the generality of this effect in nature remains unclear due to the large variability of egg shell properties, nesting sites (e.g. caves, field) and incubation patterns between species. We suggest that to disentangle the effects of asymmetric light input and head position, fMRI techniques can be successfully used to identify the head position in eggs without exposing them to light. Another possible environmental factor influencing lateralization is cradling. But, although cradling bias can potentially have an effect (Damerose & Vauclair (2002) found that right-handed mothers preferentially cradle the baby with the left arm thereby causing asymmetric input of stimuli and restraint of movement of the baby's right arm), longitudinal studies are lacking and no effect has been shown. Furthermore, it has been suggested that population level lateralization could be beneficial in social interactions, but whether social interactions influence individual lateralization is unknown. We presented preliminary data investigating this possible influence in young chicks housed in 10 groups and we showed that variation in lateralization in visually guided behaviour was larger between than within groups. This is the first evidence suggesting that lateralization of visually guided behaviours can be modulated by post-hatching social interactions. In humans, effects of social interactions can be found in overt or covert social pressure to use, for example, a certain hand. The increase of frequency of right-handers with age has been attributed to these overt and covert pressures as social pressures against left-handedness diminished over the course of recent history, although other theories

postulate that changes in the percentage of right-handers with age are caused by selective disappearance. Although the latter explanation seems less plausible (e.g. Harris 1993; Freitas & Martin 2002), no longitudinal studies exist that could differentiate between the explanations for this age-dependent phenomenon. Collins (1975) investigated the effect of covert pressures resulting from a right-biased world in mice and found that 90% of mice in a right-biased world (mimicking the right-biased world humans live in) showed a right-paw preference in contrast to no population bias in a non-right-biased world. This result came about due to a change in paw use in weakly left-pawed mice, suggesting that direction and strength of handedness are not independent factors.

Chapter two showed that the extent to which environmental factors influence lateralization may be bigger than was previously thought. However, the interaction between the genetic and environmental influence has not been explicitly investigated. More insight into this gene-environment interaction could be obtained by, for example, varying the environment in animal models in which the genetic background relevant for direction or strength of lateralization has been altered. Furthermore, longitudinal studies in human and non-human species may provide crucial knowledge of the typical development of lateralization and the effects of early environmental factors on lateralization later in life. Knowledge about the typical ontogeny of lateralization may also help explain the underlying mechanisms and functionality of atypical lateralization, like human left-handedness.

Based on the sex differences in human lateralization several hypotheses have been postulated that suggest a role in the ontogeny of lateralization for another factor: prenatal testosterone (reviewed in Pfannkuche *et al.* 2009). Although lateralization is known to be a fundamental principle of brain and behaviour in vertebrates, animal studies that could differentiate between the various hypotheses are scarce and results are ambiguous. This ambiguity may be caused by confusion between the effect of prenatal testosterone and the effect of testosterone later in life. Chapters 3 and 4 investigated the effect of prenatal and postnatal testosterone respectively.

In chapter 3 we found, by mimicking increased levels of maternal testosterone that, in a visually guided behaviour test using a predator as stimulus, prenatal testosterone affected lateralization in female but not in male cichlid fish. Control fish showed a significant population bias to view their mirror-image with their left eye and a non-significant population bias to view a predator with the right eye; both results concerning direction of lateralization are in accordance with the literature. Testosterone treated females differed significantly from control females; they showed a population bias to view a predator with the left eye, thus using the right cerebral hemisphere. Our results do not support the sexual differentiation hypothesis (Hines & Shipley 1984) as lateralization of females treated prenatally with testosterone did not shift towards that of males compared to control treated females. Furthermore, our results do not support the Geschwind and Galaburda hypothesis (Geschwind & Galaburda 1985) as no increase of right hemisphere dominance while viewing a predator was found in testosterone treated males (although this effect was observed in females) and no increase of strength of lateralization when viewing the mirror image was found. Moreover, the Geschwind and Galaburda hypothesis is based on sex differences in humans, whereas no significant sex differences were

observed in the control cichlid fish. The callosal hypothesis (Witelson & Nowakowski 1991) was not considered as fish have no anatomic structure homologous to the corpus callosum. As we found an effect of prenatal testosterone on females only, we suggested that mothers can influence the lateralization pattern of their daughters but not that of their sons.

Next, in chapter 4, we investigated whether testosterone also affects lateralization later in life. We found that testosterone treated fish showed a population bias to view a predator with the right eye, thus using the left cerebral hemisphere. Postnatal testosterone only affected male cichlid fish; control males did not show a population bias to view a predator with a specific eye, whereas testosterone treated males showed a population bias to view a predator with the right eye. This latter directional preference is in accordance with the literature on directional preference in viewing a predator. Strength of lateralization was not significantly affected by either prenatal or postnatal testosterone. In chapter 3 we suggested that various mechanisms may underlie the inheritance of strength and direction of lateralization. We found that prenatal testosterone affected direction, but not strength of lateralization in visually guided behaviour using a predator as stimulus in females. Strength of lateralization has been shown to be heritable in cichlids (Brown *et al.* 2007), possibly under genomic control, wheras we showed that direction of lateralization is controlled by (heritable) environmental factors such as prenatal gonadal hormones (chapter 3). Additionally, our finding that prenatal testosterone affects lateralization in one domain (predator viewing) but not in another (conspecific viewing) is in concordance with literature on humans in which prenatal testosterone correlated differently with lateralization patterns of language and handedness (Lust *et al.* 2011). Our results support the suggestion that population level lateralization has other costs and benefits associated with anti-predatory behaviours than with social behaviours. Strong population lateralization in anti-predatory behaviour could be exploited by the predator, whereas strong population lateralization in social interactions may be advantageous (Sovrano *et al.* 1999). Therefore, the combination of a high environmentally induced flexibility in the direction of lateralization when viewing predators, and a lower flexibility in the direction of lateralization when viewing conspecifics may have an adaptive advantage.

The effect of postnatal testosterone on lateralization was investigated in a rotational preference test using a predator as the stimulus. We found that, in males, lateralization could be changed well after the neonatal period (chapter 4). We speculate that within species a change in life history stage from fish being more solitary to becoming more social, inducing males to come together on the breeding grounds, making synchronous escape movements away from predators beneficial. Such an association between sociality and lateralization was also found in the interspecific variation in lateralization that correlated with variation in the tendency to shoal (Bisazza *et al.* 2000), which may also vary with predation pressure and other environmental variables (Brown & Warburton 1997).

As chapter 3 showed that maternal testosterone is able to alter the lateralization pattern of females but not of males, and chapter 4 showed that postnatal testosterone is able to alter the lateralization pattern of males, but not of females, it seems that in both

sexes lateralization is affected by testosterone, but that the underlying mechanisms are different between the sexes. This highlights that lateralization in general and the effect of gonadal hormones specifically should be investigated with respect to the sex and age of the animal.

Lateralization may also be influenced by practicing more with one side of the body than with the other. We investigated the potential effect of training on lateralization in chapter 5 in a non-industrial human population in which only a part of the population (had) attended school. In school specific motor tasks (such as writing) are practiced repeatedly with one hand. We tested the effect of schooling on hand preference and hand skill asymmetry. These two measures show only a weak correlation (e.g. Connolly & Bishop 1992; Steenhuis 1999; chapter 5), and can therefore be seen as two fairly independent aspects of cerebral lateralization. When controlled for sex and age, factors that have been shown to affect lateralization (Gilbert & Wysocki 1992; Papadatou-Pastou *et al.* 2008; chapter 5), we found that subjects who (had) attended school had a stronger right-hand preference than do individuals who had not attended school. Overall hand skill, independent of lateralization, was better in schooled individuals compared to non-schooled individuals. Although schooling predicted hand preference, it did not predict asymmetry of hand skill as was tested in three tasks: ball throwing, pegboard and hand grip force. The differences found between schooled and non-schooled individuals may be explained in two ways that are not mutually exclusive. First, schooling could affect hand preference, possibly mediated by social pressures of teachers to use the right hand for writing, or by reinforcement of initial preferences due to increased usage of the preferred hand when specialized tasks like writing are conducted. Second, going to school in this society implies that an individual does not participate in the daily routine of working in the gardens situated on steep slopes, or going up into the forests for hunting and trapping, both activities that promote the use of both hands. In other words, in addition to being an effect of more frequent unilateral use of upper limbs due to practicing a specific hand in school, the difference in hand preference between the schooled and non-schooled individuals may be caused by more frequent bilateral use in the living environment of non-schooled individuals. Since hand preference was associated with school attendance and asymmetry in hand skill was not, we conclude that asymmetry in hand skill is not the major determinant of hand preference.

In chapters two to five we clearly showed that, although lateralization may have a genetically determined aspect, it shows phenotypic plasticity both early in life (chapter 3) and well after the prenatal period (chapter 4 and 5).

Ultimate factors underlying lateralization

The second part of this thesis addressed ultimate questions concerning lateralization. Raymond and colleagues (1996) postulated an intriguing evolutionary hypothesis for the polymorphism in handedness: the fighting hypothesis. This hypothesis states that, even though there are costs associated with left-handedness (see Llaurens *et al.* 2009 and references therein), left-handedness could be maintained in populations due to a frequency-

dependent advantage for left-handers in fights. Evidence supporting this hypothesis was presented in a cross-cultural study correlating incidence of homicide with percentages of left-handedness in non-industrial societies (Faurie & Raymond 2005). However, the estimates of frequency of left-handedness in two out of the three societies with the highest homicide rates were based on indirect measures and were therefore unreliable. In chapter 6 we investigated this fighting hypothesis in more detail by measuring handedness, using 10 ecologically relevant tests, in a non-industrial population in which homicide rates were reported to be very high. The same population, the Eipo population in Papua, Indonesia, was incorporated in Faurie & Raymond's (2005) cross-cultural study, and the estimate concerning the frequency of left-handedness was based on pierced ears, an indicator for bow and arrow use, visible in photographs of men. In contrast to their estimate (20.4% left-handers) and to the expectations based on the fighting hypothesis we found very low levels of left-handedness (3.6%). As the fighting hypothesis mainly concerns the male part of the population old enough to have experienced the high presence of aggression common before the activity of Christian missionaries, we repeated the analyses for the older individuals and for men only (see chapter 6, supplementary material). However, this did not significantly change the results. The percentage of left-handers in our study significantly differed from the estimated percentage of left-handers by Faurie and Raymond (2005) and replacing their indirect estimate by our data eliminated the significant correlation in the cross-cultural study by Faurie and Raymond (chapter 8). Taken together with the probability of erroneous estimates for other populations in this study (caused by the use of indirect indicators of left-handedness), and flaws in data collection (high estimates of left-handedness among the Yanomamö were not based on the number of individuals that were left-handed – no left-handers were found, although no handedness could be statistically obtained of most individuals – but on the percentage of bouts that were performed with the left hand in tasks by all subjects added up (data in Marchant *et al.* 1995)) we conclude that the fighting hypothesis can no longer be sustained as the evolutionary driving force behind the persistence of left-handedness in humans.

Next, in chapter 7, we further investigated possible functional explanations for the existence of the polymorphism in handedness by investigating the association between fitness variables and aspects of handedness, asymmetry of hand skill and hand preference, in the non-industrial Eipo society. We found no significant associations between fitness variables and handedness in women, but we did in men. The asymmetry of hand skill as measured with the pegboard task was positively associated with the number of children born. In addition, a trend for a positive relationship between asymmetry of hand skill as measured with the pegboard task with the number of children alive at the time of the research was found. Strongly lateralized men thus had a fitness advantage over weakly lateralized men. On the other hand, in hand preference, a significant positive association was found between the strength of hand preference and the number of children that died before the age of two. We cautiously conclude that men with a relatively strong hand preference may have a fitness disadvantage due to a higher number of children that died in the first two years of life, possibly resulting in a decrease of number of children alive (although the latter relationship was not statistically significant). For men, it thus seems

to be advantageous to be strongly lateralized in hand skill, but disadvantageous to be strongly lateralized in hand preference.

Due to the low number of individuals with a left hand preference in this non-industrial population (chapter 6) we have not been able to investigate associations between direction of hand preference and reproductive success. By contrast, it was possible for us to investigate the associations between the direction of hand skill and reproductive success, since a considerable number of individuals performed better with the left than with the right hand, again stressing the weak correlation between hand preference and asymmetry of hand skill (see also chapter 5). However, in our findings concerning the relationship between asymmetry of hand skill and reproductive success, again only associations concerning strength of lateralization, independent of direction, were found.

As stated above, we found that strong lateralization in hand skill is an advantageous trait in men. However, as we still find weakly lateralized individuals in this non-industrial society, natural selection has apparently not rendered weak lateralization in hand skill extinct. This may possibly be a result of sexual antagonism, a specific form of balancing selection. Sexual antagonism may explain the persistence of traits in a population that have negative effects in one but positive effects in the other sex. Although we found no significant associations between strength of lateralization and reproductive success in women, we did find that eight out of nine associations between strength of handedness and reproductive success were opposite in direction for men and women, suggesting sexual antagonism. Natural selection favouring strong lateralization in hand skill in men may thus be counteracted due to possible detrimental effects on reproductive success in women. As men show more variation in reproductive success than women (Brown *et al.* 2009), larger sample sizes are needed to find significant associations between traits and reproductive success in women than in men. Studies including higher numbers of women may shed light on the possibility of sexual antagonism driving the persistence of different phenotypes of handedness. Additionally, the variation in handedness may be maintained due to balancing selection between the weak, but significant correlation between hand preference and hand skill asymmetry, the former having negative and the latter having positive effects on fitness in men.

In contrast to many studies showing a relationship between diseases and handedness (see references in chapter 7), we found no evidence that health issues were underlying the associations between handedness and reproductive success. This may mean that health issues played no role in these associations in the Eipo population, but there are two alternative explanations, First, individuals suffering from severe diseases may have died from these diseases due to lack of sufficient health care, resulting in selective disappearance if handedness and health issues are associated. Second, as no written records of health histories were available and we had to rely on self-reported data, we could not differentiate between different kinds of illnesses, nor objectively evaluate their severeness.

We showed that most probably selection pressures act on strength of lateralization and not on direction, as only variance in strength is correlated with aspects of fitness. Furthermore, we suggest that studies focussing on the differential fitness revenues of handedness between the sexes, and between hand preference and asymmetry of hand skill will shed more light on the mechanisms underlying the different phenotypes of handedness.

The fighting hypothesis (Raymond *et al.* 1996), the only evolutionary explanation that was postulated for the polymorphism in handedness in all human populations, concerns only the direction of hand preference and only in one sex. We have shown that this hypothesis cannot explain the persistence and variation in frequency of left-handedness (chapter 6), although the underlying idea (left-handers have increased chances to win fights) may still be valid. In chapter 6 we suggested an alternative explanation of why a low frequency of left-handers was found in this population that until recently lacked Western health care: the persistence of left-handedness may be related to health problems. In chapter 7 we investigated this in the same non-industrial society and found no such association. However, the results were possibly hampered by maladministration of diseases, or selective disappearance, and by the very low number of people with a left hand preference. As left-handedness may at least partially be a by-product of health problems, we postulate the health care hypothesis that states that frequency of left-handedness may rise when selection pressures operating against diseases diminish due to expanding health care. To examine our health care hypothesis we investigated whether Western health care may influence frequencies of left-handedness in populations as an alternative or in addition to homicide rate (the measure used in the cross-cultural study supporting the fighting hypothesis). We performed a cross-cultural study among 12 Western populations of which samples with known hand preference were available (collected by Perelle & Ehrman 1994) and public expenditure on health care and homicide rate were known. The results were presented in chapter 8. A regression analysis showed that public expenditure on health care was positively and significantly related to percentages of left-handedness in a population, supporting our health care hypothesis. Additionally, this association was significantly stronger than the association between left-handedness and homicide rate, the measure used by (Faurie & Raymond 2005) in the cross-cultural study investigating the fighting hypothesis.

McManus and colleagues (2010) found increasing levels of left-handedness since 1900. Our health care hypothesis could explain this increase as it coincides with the professionalization of public health that started in Britain around the latter half of the 19th century (Starr 2009).

In conclusion

Lateralization is most likely a polygenic trait (chapter 2) and both prenatal (chapter 3) and postnatal factors (chapters 4 and 5) can sex-specifically influence the ontogeny of lateralization. Cerebral lateralization can be measured both in terms of direction (left versus right) and strength (independent of direction), and these are only weakly correlated, creating room for differential selection on both aspects (chapter 7). Cerebral lateralization is expressed in behaviour, both in the individual's preference to use one side of the body over the other and in asymmetry of skill (chapters 5, 7). Preference and asymmetry of hand skill are also only weakly correlated and indeed seem to be under different natural selection pressures (chapter 7). This multidimensional aspect, together with sexual antagonism, could contribute to the balancing selection that is responsible for the stable polymorphism that lateralization in handedness seems to be (chapter 7). Clearly, lateralization is not a unitary entity, making understanding it more difficult but

also making the phenomenon more intriguing. The fighting hypothesis is unlikely to be the evolutionary driving force underlying the polymorphism of handedness (chapter 6). More likely, health care may contribute to the non-genetic variation between individuals and populations by affecting the development (part one of this thesis) of lateralization neonatally (e.g. via birth stress) or postnatally (e.g. via somatic injuries). Also, health care may contribute to selective disappearance of individuals with a certain lateralization pattern and thereby influencing the frequency of left-handedness in human populations (chapter 8).

References

Bisazza, A., Cantalupo, C., Capocchiano, M. & Vallortigara, G. 2000. Population lateralisation and social behaviour: A study with 16 species of fish. *Laterality*, **5(3)**, 269–284.

Brown, C. & Warburton, K. 1997. Predator recognition and anti-predator responses in the rainbowfish Melanotaenia eachamensis. *Behavioral Ecology and Sociobiology*, **41(1)**, 61-68.

Brown, C., Western, J. & Braithwaite, V. 2007. The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour*, **74(2)**, 231-238.

Brown, G.R., Laland, K.N. & Mulder, M.B. 2009. Bateman's principles and human sex roles. *Trends in Ecology & Evolution*, **24(6)**, 297-304.

Collins, R.L. 1975. When left-handed mice live in right-handed worlds. *Science*, **187(4172)**, 181–184.

Connolly, K.J. & Bishop, D.V. 1992. The measurement of handedness: a cross-cultural comparison of samples from England and Papua New Guinea. *Neuropsychologia*, **30(1)**, 13-26.

Damerose, E. & Vauclair, J. 2002. Posture and laterality in human and non-human primates : Asymmetries in maternal handling and the infant's early motor asymmetries. In L.J. Rogers & M. Andrew, eds. *Comparative Vertebrate Lateralization*. Cambridge, UK: Cambridge University Press, pp. 306-362.

Faurie, C. & Raymond, M. 2005. Handedness, homicide and negative frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, **272(1558)**, 25-28.

Geschwind, N. & Galaburda, A.M. 1985. Cerebral lateralization - Biological mechanisms, associations, and pathology: I-III A hypothesis and a program for research. *Archives of Neurology*, **42**, 428–459, 521–552, 634–654.

Gilbert, A.N. & Wysocki, C.J. 1992. Hand preference and age in the United States. *Neuropsychologia*, **30(7)**, 601–608.

Harris, L.J. 1993. Do left-handers die sooner than right-handers? Commentary on Coren and Halpern's (1991) "Left-handedness: A marker for decreased survival fitness." *Psychological Bulletin*, **114(2)**, 203-234.

Hines, M. & Shipley, C. 1984. Prenatal exposure to diethylstilbestrol (DES) and the development of sexually dimorphic cognitive abilities and cerebral lateralization. *Developmental Psychology*, **20(1)**, 81-94.

Llaurens, V., Raymond, M. & Faurie, C. 2009. Why are some people left-handed? An evolutionary perspective. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 881-94.

Lust, J., Geuze, R., Van de Beek, C., Cohen-Kettenis, P., Bouma, A & Groothuis, T. 2011. Differential effects of prenatal testosterone on lateralization of handedness and language. *Neuropsychology*, **25(5)**, 581-589

Marchant, L., McGrew, W. & Eibl-Eibesfeldt, I. 1995. Is human handedness universal? Ethological analyses from three traditional cultures. *Ethiology*, **101**, 239-258.

McManus, I., Moore, J., Freegard, M. & Rawles, R. 2010. Science in the Making: Right Hand, Left Hand. III: Estimating historical rates of left-handedness. *Laterality*, **15(1/2)**, 186-208.

Papadatou-Pastou, M., Martin, M., Munafò, M.R. & Jones, G.V. 2008. Sex differences in left-handedness: a meta-analysis of 144 studies. *Psychological Bulletin*, **134(5)**, 677-699.

Perelle, I.B. & Ehrman, L. 1994. An international study of human handedness: the data. *Behavior Genetics*, **24(3)**, 217-227.

Pfannkuche, K.A., Bouma, Anke & Groothuis, T. 2009. Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **364(1519)**, 929-942.

Raymond, M., Pontier, D., Dufour, A.B. & Møller, A.P. 1996. Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society B: Biological Sciences*, **263(1377)**, 1627–1633.

Rogers, L. 1996. Behavioral, structural and neurochemical asymmetries in the avian brain: a model system for studying visual development and processing. *Neuroscience & Biobehavioral Reviews*, **20(3)**, 487–503.

Sovrano, V., Rainoldi, C., Bisazza, A. & Vallortigara, G. 1999. Roots of brain specializations: preferential left-eye use during mirror image inspection in six species of teleost fish. *Behavioural Brain Research*, **106**, 175-180.

Starr, P. 2009. Professionalization and public health: historical legacies, continuing dilemmas. *Journal of Public Health Management and Practice*, **15(6)**, S26-30.

Steenhuis, R.E. 1999. The relation between hand preference and hand performance: what you get depends on what you measure. *Laterality*, **4(1)**, pp.3-26.

Witelson, S.F. & Nowakowski, R.S. 1991. Left out axons make men right: a hypothesis for the origin of handedenss and functional asymmetry. *Neuropsychologia*, **4**, 327-333.

10

Het hoe en waardoor van links en rechts - Ontwikkeling en functionele relevantie van lateralisatie

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Inleiding

Lateralisatie duidt op de hemisfeerspecifieke specialisatie van functies. Dit betekent dat de twee hersenhelften zich specialiseren in verschillende functies. Deze lateralisatie van de hersenen komt onder andere tot uiting in asymmetrische gedragingen. Daarbij kun je bijvoorbeeld denken aan links- of rechtshandigheid bij mensen. Hersenlateralisatie werd lang gezien als een typisch menselijke eigenschap en de oorzaak waardoor alleen de mens taal konden ontwikkelen. Nader onderzoek maakte echter duidelijk dat lateralisatie in het hele dierenrijk voorkomt. Zo blijkt dat ook bijvoorbeeld vissenhersenen gelateraliseerd zijn, hetgeen tot ook uiting komt in hun gedrag. Een voorbeeld is, dat veel vissen bij voorkeur het linker dan wel het rechter oog gebruiken om naar bepaalde stimuli te kijken.

Doordat lateralisatie blijkt voor te komen in de hersenen en het gedrag van alle gewervelden (en zelfs ook van een aantal ongewervelden), is het mogelijk om dieren te gebruiken als modellen voor de situatie bij mensen of bij andere dieren. Diermodellen kunnen helpen antwoorden te vinden op de vier fundamentele vragen die je kunt stellen in de gedragsbiologie: vragen naar oorzaak (causatie), ontwikkeling, evolutie en functie van lateralisatie. Met andere woorden: hoe werkt lateralisatie? Hoe ontwikkelt het zich in een individu? Hoe is het ooit ontstaan? En waar is het goed voor? Vaak worden de eerste twee vragen de 'proximate vragen' genoemd en de laatste twee de 'ultimate vragen'. In **hoofdstuk 1** geef ik een overzicht en inleiding tot de vraagstellingen die worden behandeld in deze dissertatie. Ik probeer een bijdrage te leveren aan het begrip van lateralisatie door zowel proximate (deel 1) als ultimate (deel 2) factoren te onderzoeken.

Lateralisatie is gedeeltelijk erfelijk. Door een wisselwerking met omgevingsfactoren kan echter één en dezelfde genetische blauwdruk tot verschillende kenmerken leiden. Deze fenotypische plasticiteit kan een belangrijke rol spelen in de ontwikkeling. In Deel 1 van deze dissertatie hoofdstukken 2 tot en met 5, onderzoek ik de ontwikkeling van lateralisatie en de factoren die daarin een rol spelen. Aangetoond is dat lateralisatie de efficiëntie van hersenen bevordert, doordat het verdubbeling van functies grotendeels overbodig maakt. Daardoor wordt neurale ruimte bespaard en kunnen de hersenen meerdere processen simultaan verwerken. Het is echter niet duidelijk hoe het komt dat het merendeel van een populatie vaak naar dezelfde kant toe is gelateraliseerd – de meeste mensen zijn bijvoorbeeld rechtshandig. In Deel 2 van deze dissertatie, hoofdstukken 6 tot en met 8, ga ik in op de meer functioneel en evolutionair georiënteerde vragen over lateralisatie.

Deel 1: De oorzaak en ontwikkeling van lateralisatie

Genetische en niet-genetische factoren spelen een rol

In **hoofdstuk 2** vatten mijn medeauteurs en ik de bestaande literatuur over de genetische basis van lateralisatie samen. Wij richten ons hierbij op links- en rechtshandigheid (kortweg 'handigheid' genoemd in de rest van deze tekst) bij mensen, aangezien hier verreweg het meeste over bekend is omdat het de duidelijkste gedragsmatige uiting van lateralisatie is. Uit de literatuur concluderen wij dat lateralisatie genetisch erfelijk is, maar dat de mate van genetische overerving niet bekend is en dat de huidige genetische modellen niet voldoen. Vervolgens bestuderen wij in hoeverre de ontwikkeling van lateralisatie in het individu beïnvloed kan worden door niet-genetische factoren, zoals onder andere leeftijd, hoofdpositie tijdens de prenatale periode en asymmetrische input van externe prikkels als licht. Ook presenteren wij in dit hoofdstuk voorlopige resultaten van een experiment dat aantoont dat sociale interacties lateralisatie kunnen beïnvloeden.

De effecten van prenataal testosteron op lateralisatie

Lateralisatie heeft twee aspecten die door genen of de omgeving beïnvloed kunnen worden: richting en sterkte. Richting is het meest in het oog springende aspect van lateralisatie en kan omschreven worden met 'links' of 'rechts'. De sterkte van lateralisatie is de mate waarin een individu afwijkt van geen lateralisatie, onafhankelijk van de richting. In hoofdstuk 3 en 4 onderzoeken wij één specifieke factor die de richting en de sterkte van lateralisatie mogelijk zou kunnen beïnvloeden. Die factor heeft te maken met sekseverschillen. Zo zijn mannen vaker linkshandig dan vrouwen, maar zijn rechtshandige mannen vaak sterker gelateraliseerd (sterker rechtshandig) dan rechtshandige vrouwen. Omdat mannen in de baarmoeder worden blootgesteld aan meer testosteron dan vrouwen, wordt er vaak aan dit hormoon gedacht als mogelijke veroorzaker van het sekseverschil in lateralisatie.

In **hoofdstuk 3** onderzoeken wij of prenatale testosteronspiegels bij vissen van invloed zijn op lateralisatie. Onze resultaten laten zien dat bij de geteste vissoort een verhoogde prenatale blootstelling aan testosteron alleen een effect heeft op de lateralisatie van vrouwtjes en niet van mannetjes, en alleen in één van de twee geteste functies. De resultaten onderbouwen geen van de bestaande theorieën over de effecten van prenataal testosteron op lateralisatie. Ons onderzoek laat onder andere zien dat moeders via de testosteronspiegel wel het lateralisatiepatroon van hun dochters kunnen beïnvloeden, maar niet dat van hun zonen.

Ook tonen wij in hoofdstuk 3 aan dat prenataal testosteron lateralisatie kan beïnvloeden in één domein (kijken naar roofvis) en niet in een andere (kijken naar eigen spiegelbeeld).

De effecten van postnataal testosteron op lateralisatie

De meeste studies, inclusief die in hoofdstuk 3 van deze dissertatie, hebben zich gericht op de invloed van testosteron op lateralisatie tijdens de prenatale periode. Het is echter goed mogelijk dat testosteron ook later in het leven van invloed is op de lateralisatie. Dit is onderzocht in **hoofdstuk 4**. In dit hoofdstuk beschrijven wij hoe we experimenteel de postnatale testosteronspiegels verhogen in net geslachtsrijpe vissen. Weer vonden wij een seksespecifiek effect van testosteron, maar nu andersom; alleen mannen reageerden op verhoogd postnataal testosteron.

In hoofdstuk 3 en 4 vinden wij seksespecifieke effecten van pre- en postnataal testosteron op lateralisatie. Wij vinden in beide studies echter alleen effecten op de richting, niet op de sterkte van lateralisatie. De ontwikkeling van de sterkte van lateralisatie verloopt blijkbaar anders dan de ontwikkeling van de richting. Alleen in de laatste speelt testosteron een rol. Bovendien suggereren de verschillen in effecten bij mannetjes en vrouwtjes, dat de werking van pre- en postnataal testosteron verschilt tussen de seksen. Beide studies laten zien dat onderzoek naar lateralisatie in het algemeen, en naar de effecten van testosteron op lateralisatie in het bijzonder, rekening gehouden moet worden met de sekse van het dier, iets wat in de meeste studies niet het geval is.

De effecten van scholing op lateralisatie

Een andere factor die de ontwikkeling van lateralisatie kan beïnvloeden is training. Het is bijvoorbeeld voorstelbaar dat een lichte voorkeur voor het gebruik van de rechterhand wordt versterkt, doordat die hand, juist als gevolg van die voorkeur, vaker gebruikt wordt. Zo kan handigheid bij mensen soms al in de baarmoeder worden vastgesteld, al is de voorkeur, als die al aantoonbaar aanwezig is, zwak. Ook externe factoren, zoals sociale druk, kunnen bijdragen aan training van de rechterhand. Dergelijke training van één kant van het lichaam, zoals de rechterhand, zou lateralisatie mogelijk op twee manieren kunnen beïnvloeden. Ten eerste kan het verschillen in prestatie versterken, bijvoorbeeld als de rechterhand steeds sterker en sneller wordt dan de linker. Daarnaast kan het de voorkeur voor het gebruik van een hand versterken. Beide mogelijke invloeden van training worden bestudeerd in **hoofdstuk 5**.

Op school wordt tijdens het leren van specialistische taken als schrijven één van de twee handen substantieel en dagelijks getraind. Scholing is daarom een handvat waarmee de relatie tussen training en lateralisatie bij mensen kan worden bestudeerd. Zulk onderzoek kan alleen uitgevoerd worden in een populatie waarvan een substantieel deel nooit naar school gaat of is geweest. De pre-industriële (of, beter gezegd, niet-industriële) Eipo-gemeenschap is één van de weinige populaties op aarde waarin dit (nog) het geval is. De Eipo-vallei ligt in de hooglanden van de Indonesische provincie Papoea – niet te verwarren met het autonome land Papoea-Nieuw-Guinea dat de andere helft van hetzelfde eiland Nieuw-Guinea beslaat. Sinds 1981 heeft de Eipo-bevolking toegang tot een lagere school, maar hoewel scholing gratis is, gaan velen er niet naartoe.

Ons onderzoek laat zien dat scholing samenhangt met handvoorkeur; mensen die minstens 1 jaar naar school zijn geweest, hebben een sterkere voorkeur om hun rechterhand te gebruiken bij verschillende taken dan mensen die nooit naar school zijn geweest. Dit verschil zou, enerzijds, een direct gevolg kunnen zijn van scholing, doordat in

school druk op de leerling wordt uitgeoefend. Die druk kan openlijk (bijvoorbeeld door de leraar) of verscholen (scharen en andere gebruiksvoorwerpen zijn vaak alleen geschikt voor rechtshandig gebruik) zijn. Of doordat door herhaling tijdens het leerproces de eigen voorkeur versterkt wordt. Anderzijds zou, naast scholing, ook een andere levensstijl het verschil kunnen veroorzaken tussen mensen die minstens een jaar scholing hebben gevolgd en mensen die nooit naar school zijn geweest. De laatste groep werkt bijvoorbeeld op steile hellingen in de tuinen of ze jagen in het bos. In dergelijke situaties wordt het gebruik van beide handen, vaak zelfs voor dezelfde taken, veel sterker gestimuleerd dan op school en dit zou een bijkomende oorzaak kunnen zijn van het gevonden verschil.

Wij vonden een relatie tussen schoolgaan en handvoorkeur. We vonden echter geen samenhang tussen schoolgaan en lateralisatie van handprestatie. Dit suggereert dat verschil in prestatie tussen de handen geen doorslaggevende factor zou zijn voor handvoorkeur; met andere woorden, dat je beter presteert met een bepaalde hand, bepaalt niet wezenlijk welke hand je bij voorkeur gebruikt.

Tot slot

Lateralisatie heeft een erfelijke component. In hoofdstukken 2 tot en met 5 laten wij echter duidelijk zien dat fenotypische plasticiteit een belangrijke rol speelt. Zowel in de prenatale fase (hoofdstuk 3) als ver na de geboorte (hoofdstuk 4 en 5).

Deel 2: De evolutie en functie van lateralisatie

De gevechtshypothese: een hypothese over het bestaan van linkshandigen

Het tweede deel van deze dissertatie richt zich op ultimate vragen en onderzoekt een specifieke uiting van hersenlateralisatie: links- en rechtshandigheid. Linkshandigen zijn aanwezig in alle menselijke populaties, maar zij zijn altijd in de minderheid. Nu is handigheid een erfelijke eigenschap en er zijn verbanden aangetoond tussen linkshandigheid en bepaalde gezondheidsproblemen. Als deze problemen er toe leiden dat zij minder bijdragen aan de volgende generaties dan is het de vraag hoe het komt dat linkshandigheid niet in de loop van de evolutie is verdwenen.

Onderzoekers hebben aan de hand van de 'gevechtshypothese' deze evolutionaire vraag geprobeerd te beantwoorden. Deze hypothese is gebaseerd op de observatie dat linkshandigen meer kans hebben om een man-tot-man gevecht te winnen, doordat zij in de populatie in de minderheid zijn. In een gevecht is er daardoor altijd maar een kleine kans om een linkshandige tegen te komen, en vechters hebben dus minder ervaring met zo'n tegenstander. Dit voordeel zien we terug in de vechtsportstatistieken: in man-tot-man vechtsporten is het percentage linkshandigen in de top van de sport zeer hoog. De gevechtshypothese stelt dus dat linkshandigheid nog bestaat, doordat linkshandigen evolutionair voordeel hebben in samenlevingen waar vechten belangrijk is.

In **hoofdstuk 6** testen wij deze hypothese. Dit doen wij door handigheid te onderzoeken in een populatie waar tot voor kort veel man-tot-man gevechten voorkwamen, vanwege stammenoorlogen en gevechten binnen gemeenschappen. Gebaseerd op de gevechtstheorie verwachtten wij dat de frequentie van linkshandigheid, in vergelijking met andere populaties, zeer hoog was. Dat was echter niet het geval; het aantal linkshandigen in de populatie was zelfs erg laag. Onze resultaten maken de gevechtstheorie als evolutionaire verklaring voor het voorkomen van linkshandigen in populaties erg onwaarschijnlijk.

Verbanden tussen handvoorkeur, handprestatie en evolutionaire fitness

In **hoofdstuk 7** gaan wij verder op zoek naar een evolutionaire verklaring voor het voorkomen van links- en rechtshandigheid en ook voor de grote variatie in de sterkte van lateralisatie. Wij richten ons hierbij niet alleen op de voorkeur voor handgebruik, maar ook op verschillen in prestatie van de handen. Dit laatste aspect zou evolutionair gezien net zo belangrijk kunnen zijn als handvoorkeur, maar wordt vaak genegeerd in onderzoeken. In dit hoofdstuk onderzoeken wij het verband tussen handvoorkeur en -prestatie en maten van fitness, zoals voortplantingssucces en gezondheid. In Westerse en andere moderne samenlevingen zijn betekenisvolle fitnessmaten lastig te verzamelen, doordat voorbehoedsmiddelen en gezondheidzorg deze maten sterk beïnvloeden. Vandaar dat ook dit onderzoek in de Eipo-gemeenschap is uitgevoerd, waar voorbehoedsmiddelen niet aanwezig zijn en pas sinds 2005 basale gezondheidszorg toegankelijk is.

Wij konden geen verbanden ontdekken tussen lateralisatie en fitness in vrouwen, maar in mannen vonden wij die wel. Onze resultaten suggereren dat mannen met een relatief lichte handvoorkeur evolutionair gezien beter af zijn dan mannen met een sterke handvoorkeur. Verder suggereren onze resultaten dat mannen die sterk asymmetrisch zijn in handprestatie evolutionair gezien beter af zijn dan mannen met meer gelijke handprestatie. Beide zijn metingen van sterkte van lateralisatie. Wij vonden geen verbanden tussen fitness en richting van lateralisatie van prestatie. Helaas bleek het onderzoeken van richting van handvoorkeur onmogelijk door het lage percentage linkshandigen in deze populatie (hoofdstuk 6). Wij kunnen dus niet aantonen dat de richting van lateralisatie (dus links- of rechtshandigheid) evolutionair belangrijk zou zijn. Wel vonden wij dat sterkte van lateralisatie een evolutionaire rol speelt.

Kan de gezondheidszorghypothese de frequentie van linkshandigen verklaren?

Wij vonden een laag percentage linkshandigen in een niet-industriële samenleving (hoofdstuk 6) waarin tot zeer recent moderne gezondheidszorg geheel afwezig was. Ook is in veel studies een verband gevonden tussen linkshandigheid en gezondheidszorg, hoewel de gevonden relaties vaak toegeschreven kunnen worden aan verschil in sterkte van lateralisatie in plaats van richting (zie hoofdstuk 7).

Wij speculeerden dat gezondheidszorg, in aanvulling op of als alternatief voor de gevechtshypothese, het percentage linkshandigen in een populatie kan beïnvloeden. Dit hebben wij onderzocht in **hoofdstuk 8**, een literatuurstudie naar twaalf westerse landen waarvan de percentage van linkshandigen bekend zijn, evenals de publieke uitgaven voor

gezondheidszorg en het aantal opzettelijke gedode individuen (moord/doodslag). Deze laatste maat werd gebruikt door de opstellers van de gevechtshypothese. Hoofdstuk 8 laat zien dat de hoogte van publieke uitgaven voor gezondheidszorg, in tegenstelling tot het aantal opzettelijke gedode individuen, significant en positief gecorreleerd is met het percentage linkshandigen in populaties. Deze bevinding ondersteunt dus onze hypothese dat een laag niveau van gezondheidzorg, zoals in de Eipo-populatie, kan leiden tot weinig linkshandigen in een populatie.

Conclusies

Lateralisatie is een erfelijke eigenschap waarin hoogstwaarschijnlijk meerdere genen een rol spelen (hoofdstuk 2). Zowel prenatale (hoofdstuk 3) als postnatale factoren (hoofdstuk 4 en 5) kunnen, seksespecifiek, de ontwikkeling van lateralisatie bij een individu beïnvloeden. Lateralisatie van bepaalde vormen van gedrag kan enerzijds gemeten worden als voorkeur voor het gebruik van een bepaalde kant (hand, oog, etc), anderzijds als verschil in prestatie tussen links en rechts. Deze twee maten hangen slechts licht met elkaar samen en lijken op verschillende manieren beïnvloed te worden door natuurlijke selectie (hoofdstuk 7). Dit verschil in selectiedruk zou, samen met verschillen in evolutionaire voordelen van lateralisatie tussen mannen en vrouwen, kunnen verklaren waardoor verschillende varianten van lateralisatie naast elkaar kunnen blijven bestaan (hoofdstuk 7).

Het voordeel dat linkshandigen hebben in gevechten lijkt op dit moment niet, zoals voorgesteld in de gevechtshypothese, de evolutionaire kracht te zijn achter het voortbestaan van linkshandigheid in populaties (hoofdstuk 6). Gezondheidszorg lijkt een waarschijnlijker kandidaat. Gezondheidszorg zou kunnen bijdragen aan de variatie tussen individuen en populaties door direct de prenatale en postnatale ontwikkeling in het individu te beïnvloeden. Daarnaast zou gezondheidszorg natuurlijke selectie kunnen tegenwerken en daarmee de frequentie van linkshandigheid in de populatie kunnen beïnvloeden, bijvoorbeeld door de behandeling van ziektes die verband houden met linkshandigheid (hoofdstuk 8).

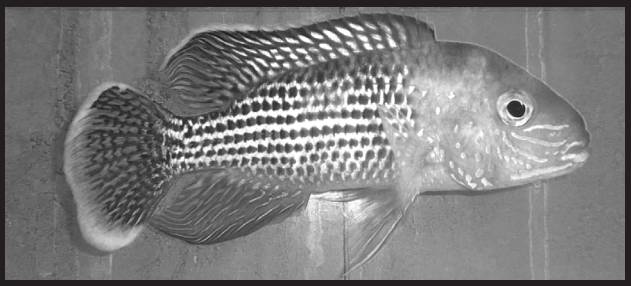


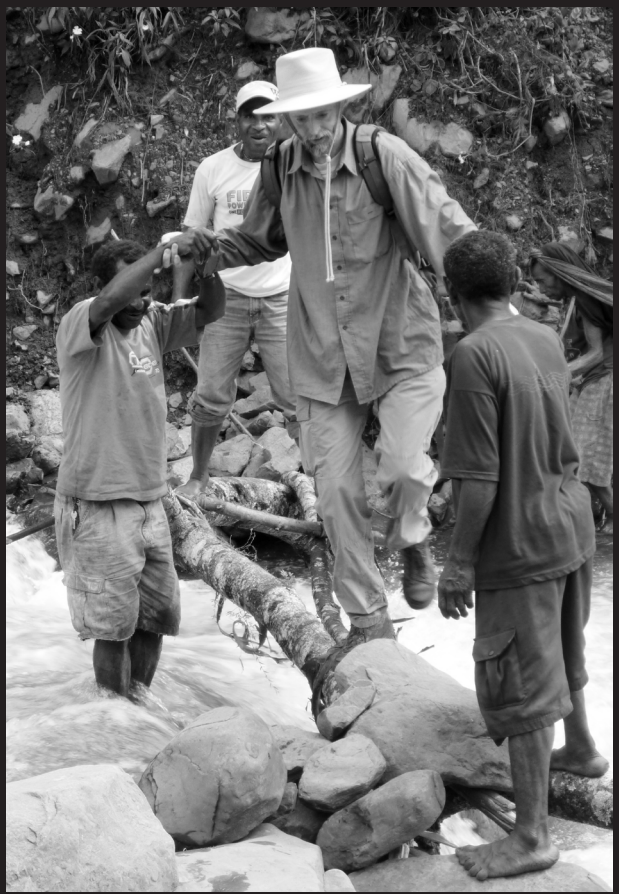
Photo: Alex Verkade

Photo: Julia Groothuis

Photo: Vivian Goerlich

Photo: Kristin Schubert

Acknowledgements



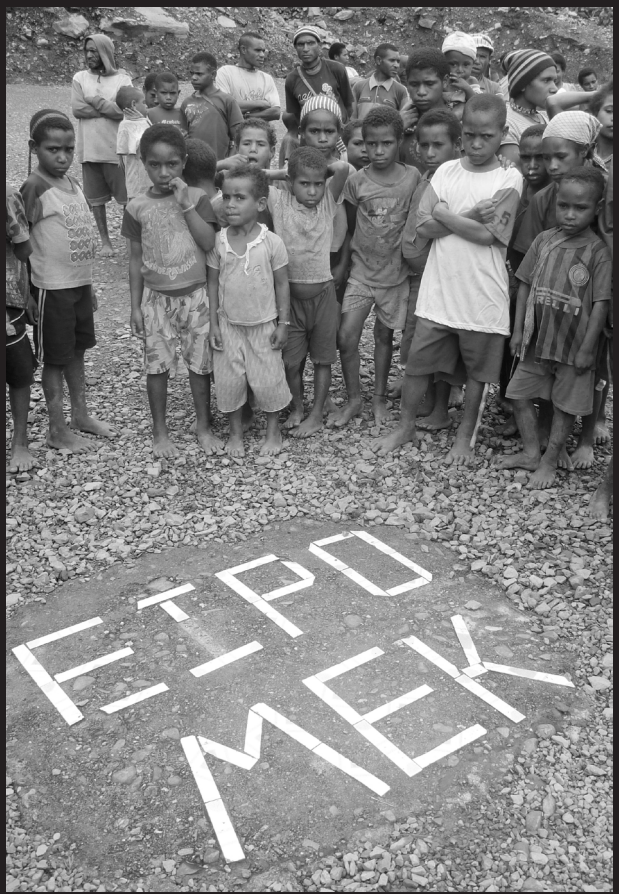


Photo: Nellie Konijnendijk

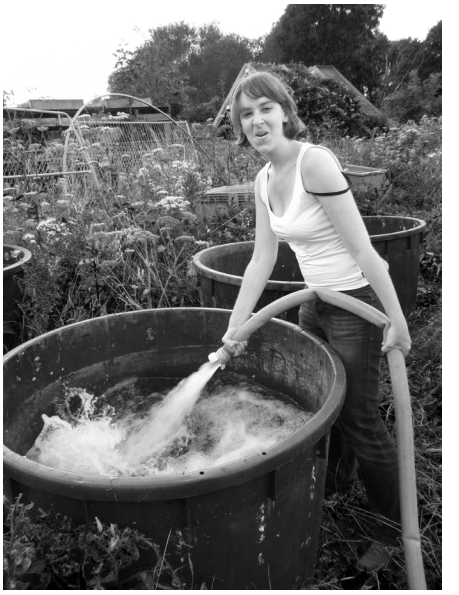


Photo: Alex Verkade

Photo: Alex Verkade

Photo: The butcher in Dornumersiel

Het is bijzonder om een hoofdstuk te mogen vullen met het bedanken van personen die belangrijk waren in deze periode van mijn leven. Allereerst wil ik graag mijn promotor bedanken, zonder wie ik nooit was begonnen aan dit avontuur. Dankzij Ton had ik de luxe om, voordat ik aan dit project begon, uitgebreid een kijkje in het lab te kunnen nemen in de vorm van een doctoraalstage. Al snel werd het mij duidelijk dat ik me hier wel thuis zou voelen. Ton, bedankt voor je vertrouwen, enthousiasme en je altijd scherp analytisch oog. Dat je wetenschap ook heel goed kunt bedrijven onder het genot van een pizza was een wijze les. Annet en Julia bedankt voor jullie gastvrijheid en het begrip dat jullie opbrachten wanneer ik weer eens (een gedeelte van) Tons avond of weekend inpikte.

Vivian, without your good care I might not have started this job; thanks for showing me around that first period and explaining me the lab's customs and habits. Even though I had to collect pigeon poo on Sunday mornings, I really enjoyed that time. Obviously I took my revenge when you had to assay the aquarium-house-incubated human saliva! Peter, dank je wel dat jij me wilde inwijden in de vissenbusiness. Mooi dat het zelfs, in combinatie met vele jaren practicum, nog een artikel heeft opgeleverd! *Kristina, we began our appointment on the same day, on the same topic and in the same cupboard... eh... office. Getting to know the ins and outs of lateralization and the lab without you wouldn't have been the same and definitely not as much fun.* Bernd, ook jij was belangrijk in deze fase van mijn onderzoek. Bedankt voor alle interessante discussies die we hebben gevoerd en je altijd frisse, uit het leven gegrepen kijk op wetenschap en andere zaken.

Ergens rond het einde van 2007 kreeg ik de mogelijkheid om naast mijn bestaande onderzoek bij vissen, onderzoek te gaan doen naar lateralisatie bij mensen. Ik zou daarvoor afreizen naar de Eipo-gemeenschap in de hooglanden van Papua, Indonesië. Deze unieke kans heb ik uiteraard met beide handen aangegrepen. Dit project, in eerste instantie bedoeld als een klein deelproject, is gaandeweg uitgegroeid tot een substantieel deel van het onderzoek beschreven in deze dissertatie. Het opzetten van een project dat uitgevoerd moet worden in een onbekende omgeving vergt veel inspanning. Ton, bedankt voor al je kennis en tijd die je (ook) in dit project gestoken hebt. Wat goed dat je de tijd vond om mee te gaan naar de Eipo vallei. Wetenschappelijke discussies zijn toch wel veel leuker als je ze voert in afgelegen oorden. Bedankt ook voor het niet nog erger uit de hand laten lopen van de hoeveelheden foto's die ik wilde houden. Reint, wat hebben we veel meegemaakt: de mooie feesten, de soms wel eeuwig durende uren in de kerk, het meisje op de berg, de prachtige wandelingen, de vele proefpersonen en hun vaak fantastische verhalen, het was een reis en onderzoek om nooit meer te vergeten. De voorbereidingen, de reis zelf en de discussies achteraf; bedankt voor al je tijd, interesse en geregel! *Besides Ton and Reint, Wulf was essential for the prosperity of this complicated project. Wulf, thank you so much for the chance you gave me to visit the Eipo society. Your knowledge, broad interest, linguistic talent and insight into the human nature are extraordinary and vital to arrange such an amazing trip and make it into a big success.* Anke, bedankt voor je vertrouwen en je input tijdens de soms ellenlange discussies, jouw kennis over lateralisatie was van hoge toegevoegde waarde. *Toni, although we met only briefly, your presence made a difference.* Uiteraard gaat mijn dank uit naar de mensen die hebben deelgenomen aan ons onderzoek, de mensen die zo openhartig hun wereld aan ons hebben laten zien en de mensen die zorgden dat we ons thuis voelden zo ver weg van huis. Extra dank gaat uit naar de drie assistenten die

ons op allerlei vlakken tijdens ons onderzoek hebben bijgestaan, zoals tijdens het scouten van proefpersonen, het vertalen naar het *Eipo yupe*, het dragen van spullen op onze tochten, het regelen van varkens voor feesten en het beschikbaar stellen van huizen en kerk tijdens ons verblijf: Philipus Nabyal, Enus Kona en Yundinus Nabyal. *Terima kasih banyak kepada seluruh masyarakat Eipo! Saya berterima kasih khusus kepada Philipus Nabyal, Enus Kona en Yundinus Nabyal. Terima kasih untuk kepanduan subyek, terjemahkan ke Eipo yupe, membawa barang-barang kami, menurus babi untuk pesta dan penyediaan rumah rumah dan gereja selama kami tinggal.*

Ook terug in Nederland zijn er natuurlijk talloze mensen die een bijdrage hebben geleverd aan deze dissertatie. Ten eerste wil ik graag Maarten Lasthuizen postuum bedanken. Hij heeft mij veel werk uit handen genomen met het meten van hormoongehaltes in de vele monsters van visseneieren. Ook wil ik Bonnie en Ilse bedanken voor hulp in het lab, Gerard voor, tja hoe noem je dat, van alles en nog wat en Marlies voor al het papierwerk (en pauzes). Roelie en Sjoerd, heel erg bedankt voor alle gezelligheid op het BC en daarbuiten. En natuurlijk bedankt voor alle goede zorg die jullie aan mijn visjes hebben gegeven! Dat geldt uiteraard ook voor Saskia, Monique en Christa. Ook heb ik tijdens verschillende projecten hulp gehad van studenten. Dafne P, Vibeke B, Anne G, Jessica T, Mendel K, Lotte O, Jasmijn G en vooral Floor B en Daan B, dank jullie wel! *I would also like to thank the members of my reading committee Prof. dr. Bisazza, Prof. dr. Koolhaas and Prof. dr. McManus for their time reviewing this dissertation.*

Besides all the scientific fun, there was some time available for the non-scientific side of life too. Kristina, I couldn't have imagined that besides working in the same 'office' we would additionally and voluntarily start to play various types of sports, go out regularly and even start living together ánd that we would have so much fun doing all that. There would be no one better suited to being my paranimf. Martijn, Ralf, Kristin, Kristina, Vivian, and in a later stage also Esther, I wouldn't have missed the countless nights in the Uiltjes, the many (not-)dancings at all kinds of places and the weekends away for the world! My time in Groningen would have been a lot less fun without you all being there too. Dikke knuffel! *Also others not yet mentioned contributed to a very nice working atmosphere. Among them: Albert, Betka, Berber, Christina, Cor, Egbert, Ellis, Els, Gert, Giulia, Jean Christophe, Jelle, Jessica, Lobke, Martina, Martine, Michael, Mirre, Rie, Sandra, Simon and Sophie.*

Al heb ik natuurlijk het grootste deel van mijn tijd als promovendus doorgebracht in Groningen, Amsterdam bleef trekken door de vele leuke mensen daar. Allert, Anne, Bart, Eric, Femke, Jessica, Jurgen, Laurens, Marieke, Nellie, Nick, Peter, Roos, Rosanne, Vicencio: popjes, wat is het fijn om jullie als vrienden te hebben! Nel, het was erg leuk om samen met jou een experiment te doen. Het heeft even geduurd, maar ik moet nog steeds lachen als ik bedenk dat ze onze 'aquaria' te laat bezorgden omdat ze meer tijd nodig hadden om, verrassing, er gaten in te raggen. Jammer dat ik ons werk niet hierin heb kunnen presenteren. Hopelijk komt dat snel. Roos, het was fijn nog een Amsterdammer in het hoge noorden te hebben. Marieke, met jou leerde ik op eigen begen te staan. Al dacht onze onderbuurman daar vast en zeker het zijne van, het was mooi! Dank je wel dat je, nu ik weer een nog grotere meid word, mijn paranimf wil zijn. Popjes, bedankt!

Ook was het fijn om zondagmiddag bij Brouwerij 't IJ even op te kunnen laden voor de nieuwe werkweek. De altijd relaxte sfeer bij 't IJ, de festivals, dagjes en weekendjes weg waren meer dan welkom in deze vaak hectische periode. Annelies, Diederik, Esther, Gerben, Jelle, Jeroen, Joost, Mark, Martine, Paul, Rudie, Sandra, Yuri en alle anderen bedankt!

Mijn laatste dagen sleutelen aan dit boekje heb ik mogen doorbrengen bij De Praktijk. Caspar, Charlotte, Miranda, Patty, Tanja en vooral Alex en Yuri bedankt voor jullie gastvrijheid en allen voor de gezellige lunches!

Ook wil ik heel graag Basje, Hassan, Ger, Marja en ook Hans en Jansje bedanken. Hoe ouder ik word, hoe vaker we elkaar zien en daar ben ik heel erg blij mee! Margreet, dank je wel voor je onvoorwaardelijk vertrouwen en enthousiasme voor alles wat ik uitspook. Je bent een voorbeeld voor mij en zal dat altijd blijven. Je moet weten dat ik vaak opschep over hoe jij de wereld aanpakt.

En dan is er natuurlijk nog Alex. Opeens verhuisde ik naar hoge noorden en liet jou achter. Dank je dat je mij daarin altijd hebt gesteund; voor de uren die je voor mij in de trein hebt gezeten, voor het aanpassen van je vakanties aan mijn conferenties en vage planningen en voor het assisteren bij het onderzoek in Papua, maar ook voor het redigeren van tekst, je hulp bij het lay-outen van dit boekje en voor zoveel meer! Dank je wel, je bent fantastisch.

En nu samen op naar het volgende avontuur.

*Telebe, agonum lebman**

* *Thank you, I have said enough.*

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